# Aspects of Swimming Physiology and Behaviour

## Consequences for Migrating Fish

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A naturalist’s life would be a happy one if he had only to observe and never to write.

*Charles Darwin*

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## ***Samenvatting***

Vissoorten migreren tussen verschillende types van habitat om hun fitness te vergroten. Deze bewegingen hoeven geen verandering van biotoop te impliceren om in een verandering van de fitness te resulteren. Aangezien diadrome migraties van grote soorten (vb. zalmachtigen, paling...) altijd de voorbeelden voor mobiliteit zijn geweest en de niet-diadrome soorten als sedentair werden beschouwd, zijn deze laatsten grotendeels uitgesloten geweest van beheersmaatregelen die naar herstel van vrije migratie van vissen in riviersystemen streven. Nochtans zijn er een massa bewijzen dat vele 'sedentaire' zoetwatervissen potamodrome migraties ondernemen.

De ruimtelijke mobiliteit dient een hele reeks doeleinden (ontogenie, schuilen, predatie, paaien), en laat het ook de rekolonisatie van habitats toe. De vrije mobiliteit van individuele vissen is niet alleen noodzakelijk voor kolonisatieprocessen, maar ook voor het handhaven van een dynamisch evenwicht binnen gemeenschappen door middel van paaimigraties, waardoor populaties worden gestabiliseerd. Habitatfragmentaties resulteren in een minder efficiënte populatiegrootte, verminderde variabiliteit en heterozygotie, hogere kansen voor genetische afwijkingen en bottlenecking, verhoogde inteelt en verminderde fitness. Al deze factoren kunnen het overleven op lange termijn compromiteren. Daarom is de mobiliteit tussen habitats een zeer belangrijke component van evolutieve processen die tot speciatie en genetische diversiteit leiden.

De toename van menselijke activiteit heeft een hele reeks van effecten op het milieu. Rivieren zijn uitgebaggerd en hun oevers zijn gewijzigd. Dammen en waterkeringen leiden tot het verlies van essentiële habitats. Deze barrieres kunnen voor migrerende vissen onoverwinbaar zijn en kunnen daarom tot een lokale extictie van populaties leiden, maar ook tot de verstoring van gehele visgemeenschappen en het uitsterven van soorten. In België zijn alle vissoorten

die over lange afstanden migreren in de loop van de afgelopen twee eeuwen in de Maas uitgestorven (geweest) en in Vlaanderen zijn vijf migrerende soorten die van de oorspronkelijk 13 overgebleven zijn, zeer zeldzaam. Dammen en waterkeringen hebben naast hun direct blokkerend effect een reeks van bijwerkingen door het wijzigen van hydrodynamische of andere fysico-chemische aspecten, die de vismigratie kunnen beïnvloeden en de timing van migratie en seizoengevoeligheid veranderen. Voor soorten met een lagere zwem- en springcapaciteit kunnen kleine hindernissen hetzelfde effect hebben als dammen en waterkeringen. Ze zijn ook de oorzaak, samen met habitatverlies en fragmentatie, voor de daling van de populaties en de het uitsterven van volledige vissoorten. Om het probleem van habitatfragmentatie op te lossen werden bij grote dammen vistrappen geintroduceerd die stroomopwaartse migratie van vissen en meer recent ook hun stroomafwaartse bewegingen moeten mogelijk maken. De geschiktheid van vistrappen hangt af van hun aantrekkelijkheid voor de migrerende vissoorten en van hun haalbaarheid, d.w.z. of de vistrap met succes door bepaalde soorten doorkruist kan worden.

Migratie, gedefinieërd als de beweging van één habitat naar een ander, is zeer afhankelijk van het zwemgedrag en de energiemetabolisme. De migratie-capaciteit berust daarom op de integratie van voortbewegingsactiviteit en de bijbehorende energievoorziening. Zoetwatervissen hebben een brede waaier van lichaamsvormen en zwemstrategieën. De resulterende diversiteit in het zwemgedrag variërt van de trage, kronkelige palingen en prikken tot de buitengewone acceleratiecapaciteit van snoekachtigen, en indrukwekkende continue zwemprestaties van diadrome migratoren zoals zalmachtigen.

**De hoofddoelstelling van deze thesis is het verhogen van de kennis over zwemgedrag, zwemprestatie en energiehuishouding van migrerende en niet-migrerende vissoorten die door de mens gemaakte hindernissen moeten overwinnen.**

Na een algemene inleiding over fysiologische, energetische en gedrags-aspecten van het zwemmen in **Hoofdstuk Eén**, presenteer ik in **Hoofdstuk Twee** een methodologisch studie over de effecten van de grootte van zwemtunnels op de kritische zwemsnelheid ($U_{crit}$) bij de karper (*Cyprinus carpio*, L.). De resultaten laten zien dat in het labo bereikte resultaten van $U_{crit}$ testen met voorzichtigheid moeten worden geïnterpreteerd. Karpers kunnen hun zwemgedrag aanpassen aan de lengte van zwemtunnels. De resultaten laten zien dat de lengte van zwemtunnels de bereikte $U_{crit}$ waarden beïnvloedt omdat de 'burst-and-glide' periode in een langere tunnel verlengd is. Daarom kunnen vissen in langere tunnels meer tegen hogere snelheid zwemmen voor ze uitgeput raken. Dit suggerereert dat het effect van de tunnellengte op het zwemgedrag niet primair beinvloedt woord door fysiologische factoren maar gewoon door plaatsgebrek. Daarentegen zijn fysiologische factoren ook betrokken, vissen gebruiken immers niet de gehele lengte van de tunnel in de 'burst-and-glide' modus. Het effect van lichaams- en tunnellengte kan daarom tot variaties in $U_{crit}$ in de bestaande literatuur bijdragen. Ook kan het niveau van het aerobe potentieel overschat worden, omdat in lange tunnels de anaerobe 'burst-and-coast' periode verlengd wordt. De snelheid waabij de overgang van aeroob naar anaeroob zwemgedrag gebeurde (gait transition) was niet beinvloed door de lengte van de zwemtunnel. Daarom kan 'gait transition' een betere referentie zijn om het zwemgedraag te testen.

**Hoofdstuk Drie** presenteert een vergelijkende studie over migrerende (trachurus) en niet-migrerende (leiurus) morphotypes van de dreidoornige stekelbaars (*Gasterosteus aculeatus* L.) en zijn energetica en zwemgedrag. Migrerende vissoorten zoals de driedoornige stekelbaars (*Gasterosteus aculeatus* L.) tonen grote veschillen in zwemcapaciteit en energetica tussen de migrerende (trachurus) en de niet-migrerende (leiurus) vorm. Vergelijkende studies over zoetwater vismigratie moeten vaak met seizoenale en tijdelijke factoren rekening houden die het moeilijk maken om de resultaten te vergelijken. De driedoornige

stekelbaars is een goed model voor het bestuderen van migrerende en niet-migrerende vormen in hetzelfde habitat en dezelfde tijd. In tegenstelling tot de Noord Amerikaanse stekelbaarzen met meer uithoudingscapaciteit in de migrerende vorm en meer burst capaciteit in niet-migrerende vorm, is de trachurus vorm in zowel lange afstands zwemmen als ook burst zwemmen beter dan de leiurus vorm. Dit laat zien dat de Europese trachurus vorm goed is aangepast aan lange diadrome migraties. De migratie naar paaigebieden vraagt om stroom opwaards te zwemmen. Daarom kan aangenomen worden dat vissen met de voor hen energetisch meest voordelige zwemsnelheid, de $U_{opt}$ proberen te zwemmen. De resultaten laten zien dat de $U_{opt}$ significant hoger is in trachurus dan in leiurus. Dit laat de conclusie toe dat trachurus sneller kan zwemmen met realtief lagere energieverbruik. Absoluut gezien verbruikt trachurus tijdens het zwemmen met $U_{opt}$ hogere hoeveelheden zuurstof, maar relatief gezien is de percentuele verhoging vergeleken met het potentieel voor aerobe activiteit dezelfde als bij leiurus. Ook de $CoT_{opt}$, de kosten van verplaatsing bij optimale zwemsnelheid, waren niet significant verschillend. Door het veranderen van de standaard metabole activiteit (SMR) kunnen migratoire soorten de aerobe activiteit (AMR) en het potentieel voor aerobe activiteit verhogen.

In **Hoofdstuk Vier** presenteer ik een geïntegreede studie over de zwemcapaciteit van zeven europeese zoetwatervissen met verschillende adaptaties voor migratie. (forel *Salmo trutta fario*, baars *Perca fluviatilis*, rietvoorn *Rutilus rutilus*, karper *Cyprinus carpio*, grondel *Gobio gobio*, rivierdonderpad *Cottus gobio*, bermpje *Barbatula barbatula*). Kritische ($U_{crit}$), optimale ($U_{opt}$) en maximale zwemsnelheid ($U_{max}$) en zuurstofverbruik ($MO_2$) werden geanalyseerd. De resultaten laten zien dat forel, rietvoorn en baars excelente zwemmers zijn, en bermpje en rivierdonderpad niet. Dit is niet verbazend omdat deze eerste soorten typische lange afstands migratoren zijn, en de laatsten niet. Maar ook de grondel, een niet-migrerende soort, zwom verbazingwekkend goed. Grondels zijn vissen die op de bodem leven en een zeer gelimiteerd migratiegedrag

vertonen. Nochtans is het potentieel voor aerobe activiteit in deze soort laag. Dit laat de conclusie toe dat een grote deel van $U_{crit}$ door anaerob metabolisme gevoed wordt. Daarom kunnen de hoge zwemsnelheden niet over een lange periode volgehouden worden.

Zwemperformantie en energieverbruik verschillen van soort tot soort. Daarom moeten verschillende potamo- en diadrome soorten beschouwd worden als men effecten van barrieres op migratie wil evalueren en moeten soorten zoals de rivierdondrpad mee in rekening gebracht worden als nieuwe vistrappen worden gebouwd. Zelfs kleine barriers kunnen immers een hinder zijn voor het uitwisselen van genetisch material tussen populaties van vissen die migreren over korte afstanden zoals bij het bermpje en de rivierdonderpad.

**Hoofdstuk Vijf** bespreekt biomechanische zwempatronen en de beweeglijkheid van drie verschillende manieren van zwemmen. De beweeglijkheid van zwemmende vissen is gedefinieerd als de minimum 'turning radius' per zwemsnelheid en is afhankelijk van de lichaamsbeweging en de flexibiliteit. Anguilliforme (paling, *Anguilla anguilla*), subcarangiforme (zeebrasem, *Abramis brama*) en labriforme zwemmers (gestreepte brandingsbaars, *Embyotoca lateralis*) tonen ieder afnemende lichaamsflexibiliteit en gebruiken een kleiner deel van hun lichaam voor de voortbeweging. Als men de laagste turning radius tegen de zwemsnelheid plot, zijn de datapunten gelijkmatig verdeeld maar met een lagere lijn waaronder geen punten aanwezig zijn. Deze lage lijn is positief gecorreleerd met zwemsnelheid en de resulterende helling van deze limiet neemt af met toenemende undulatie van de lichaam tijdens het zwemmen. Dit laat ons toe te besluiten dat de draaibewegingen minder scherp worden bij hoge snelheden in labriforme zwemmers in vergelijking tot de flexibele anguilliforme zwemmers, en dat de manouvreerbaarheid bij hoge snelheden daardoor wordt beperkt.

In **Hoofdstuk Zes** presenteer ik een studie over de spontane zwemactiviteit van de labriform zwemmende gestreepte brandingsbaars *Embyotoca lateralis*. De bedoeling van deze studie was een kinematische variable te vinden die

geassocieerd is met het zuurstofverbruik tijdens labriform zwemmen. Kinematische variabelen (zwemsnelheid, snelheidsveranderingen, hoek en radius van draaibewegingen en de frequentie van de pectorale vinbewegingen) en $MO_2$ van spontaan zwemmen werden in een ronde respirometer gemeten met behulp van video tracking. Bij deze labriforme zwemmer correleert bij lage snelheden de zuurstofopname niet met zwemsnelheid, acceleratie, en andere kinematische factoren maar met de frequentie ($f_P$) van de pectorale vinnenbewegingen. De resultaten suggereren dat de energiebehoefde van een labriform zwemmende vis accuraat kunnen ingeschat worden ($r^2 = 0.71$) als men $f_P$ gebruikt. Dit kan in de toekomst gebruikt worden om de metabole snelheden van labriforme vissen in de vrije natuur in te schatten met behulp van methoden zoals EMG en telemetrie, die de $f_P$ in het veld meten.

**Hoofdstuk zeven** bediscussieert het effect van een chemische barriere op zwemgedrag en predatie. Fast starts, snelle zwembewegingen, worden gebruikt om te prederen maar ook om een predator te ontvluchten en zijn daarom ecologisch gezien belangrijke bewegingen. Fast starts worden voortgebracht door glycolytische spieren en worden door vele interne en externe factoren beinvloedt. Het is al bekend dat ammonia vervuiling in het water een groot effect heeft op de bewegingen van witte spieren, en dus een omgeving kan creëren die fast starts reduceert en predatie belemmert. Daarom werden vlucht en predatie fast starts van forellen (*Salmo trutta*) onderzocht na blootstelling aan van een verhoogde (1 $mg.l^{-1}$) ammonia concentratie. Verschillende bewegings- en gedragsvariablen werden gemeten. Deze studie laat zien dat ammonia vooral de fast start snelheid reduceerde en de richting van het vluchtgedrag veranderde. Predatie, sociale interacties en predator-prooi relaties veranderden en het aantal gevangen prooien was gereduceerd. Het effect van ammonia was duidelijker in grote dan in kleine vissen. Als vissen door een zone met verhoogde ammonia concentraties migreren wordt fast start capaciteit en anaerobe zwemcapaciteit

gereduceerd. Dit kan leiden tot een lagere zwemsnelheid, verminderd succes bij het ontwijken van predatoren, en een gereduceerde energieopname.

Algemeen kan besloten worden dat er vele factoren zijn die de capaciteit van vissen om door de mens gemaakte barriers te overwinnen beinvloedt. Deze thesis beschrijft sommige aspecten van de zwemfysiologie en energetica betrokken bij de migratie van een aantal vissoorten. Gebaseerd op onze resultaten, zouden toekomstige studies snelheden en energieverbruik in het veld moeten meten. Ook moeten meer relevante testen om zwem- en migratiecapaciteit te meten onder gecontroleerde laboratorium-omstandigheden ontwikkeld worden. $U_{crit}$ testen blijken immers relatief ongeschikt als men de fysiologische en ecologische aspecten van zwemcapaciteit wil vergelijken. Meer onderzoek moet gebeuren naar de effecten van omgevingsfactoren op migratie, meer bepaald abiotische factoren zoals waterkwaliteit, saliniteit, pH en temperatuur, maar ook biotische factoren zoals soortensamenstelling en ziekten. Meer in het bijzonder zie ik twee gevaren die onze reeds bedreigde vissoorten verder bedreigen: opwarming van de aarde en bio-invasie. Deze aspecten zouden moeten opgenomen worden in verdere studies, die van vitaal belang kunnen zijn om migrerende soorten beter te beschermen.

## ***Summary***

Fish species migrate between different types of habitats in order to enhance their fitness. Also, the term 'migration' applies to individual fishes since it increases or decreases their fitness. However, these movements do not need to involve journeys between biomes to result in a change of the fish's fitness. As diadromous migrations of large fish (e.g. salmonids, eels…) have been the examples for mobility and non-diadromous fish species have been deemed to be resident, they have been largely excluded from management schemes aiming at the restoration of the free circulation of fishes in river systems. However, there is a bulk of evidence that many 'resident' freshwater fish species undertake potamodromous migrations.

As spatial mobility in fish serves a series of purposes (ontogenetic, refuge, feeding and spawning migrations), it also enables the recolonisation of habitats. Free mobility of individual fishes is not only necessary for colonisation processes, but also for maintaining a dynamic equilibrium within the fish community by means of spawning migrations and thus stabilizing populations. Habitat fragmentation results in lower effective population sizes, reduced variability and heterozygosity, higher chances for genetic drift and bottlenecking, increased inbreeding and hence reduced fitness, which compromises long-term survival. Therefore, mobility between habitats is a key component of evolutionary processes that lead to speciation and genetic diversity.

The increase of the human activity has resulted in a series of impacts on the environment. Streams have been dredged and their banks have been modified. Dams and weirs have been erected, resulting in a loss of essential habitats. These obstructions can be impassable for migrating fish and therefore can lead to local extinction of populations, extinction of species and disturbance of communities in the upstream reaches. For example in Belgium, all long-distance migrating fish species have been extinct over the past two centuries in the River Meuse (now improving), and in Flanders are only five of the 13 long distance migrating

species left, being very rare. Weirs and dams, in addition to their direct blocking effect, have a series of side effects, due to modifications of hydrodynamical conditions or other physico-chemical aspects, which may influence fish migration, depending on timing of migration and seasonality. For species with lesser swimming and leaping capacities, small obstacles can have the same impact as dams and weirs, and they also have been the cause, together with habitat loss and fragmentation, for the decline of populations and the thread of extinction of entire fish species.

Most efforts to solve the problem of habitat fragmentation due to the obstruction of migration routes was the introduction of fish passages over huge dams during upstream movements of migrating fish species, and more recently in their downstream movements. The adequacy of fish passages depends on their attractiveness to the migrating fish species, minimising time and energy wasted before migrants enter the fish passage, and on their feasibility, i.e. whether the fish passage can be successfully crossed by targeted species.

Migration, defined as the movement from one habitat to another, is very much depending on swimming behaviour and energetics. The capacity for migration therefore relies on the integration of locomotor activity and associated energy provision. Freshwater fish have a wide range of body forms, swimming strategies and oxygen uptake. The resulting diversity in swimming modes and performances vary from sluggish, serpentine eels and lampreys to the extraordinary acceleration ability in fast starts of esocids, and the impressive sustained swimming performance of diadromous migrators such as salmonids.

**The main objective of this thesis is to increase the knowledge on swimming behaviour, performance and the energetics of fish swimming, related to the capability of migrating and non-migrating fish species to clear man made obstruction on migration paths.**

I will try to highlight some aspects of fish migration and the passage of obstacles. Also, fish swimming patterns and energetics will be discussed. After a general introduction and presentation of physiological, energetic and behavioural aspects of fish swimming (**Chapter One**), I present in **Chapter Two** a methodological study of the effect of swimming tunnel size on critical swimming speed in common carp (*Cyprinus carpio, L.*). The results show that when testing swimming capacity in the laboratory in order to estimate migration capabilities using an $U_{crit}$ test, the results should be interpreted with care. Carp can control its behaviour during the swimming test depending on flume length. The results show that the length of the swimming tunnel has an effect on the reached critical swimming speed because of an increased burst-and-glide period after gait transition in longer swimming tunnels. Therefore fish in the longer chamber could perform much more work before becoming exhausted. This suggests that the effect of chamber length on performance is not primarily limited by physiological factors determining fatigue, but rather space constraints limiting the ability to execute the behaviour. Nevertheless, physiological factors undoubtedly also contribute to performance. Fish do not use the whole length of the swimming tunnel when burst-and-gliding after gait transition, leading to smaller distances swum. Therefore, general size-dependent effects of chamber length will be factors adding to effects of speed increment and time interval on variation in $U_{crit}$ in the published literature. Similarly, if $U_{crit}$ is considered to be the speed which maximizes oxygen uptake, longer periods of bust-and-coast swimming probably using anaerobic metabolic pathways might underestimate $\dot{V}O_{2max}$ for fish swimming in longer chambers. As it is shown here, the gait transition was not affected by the different swimming chamber lengths. Therefore, such gait transitions could be a better reference for the comparison of metabolic performance among species.

**Chapter Three** presents a comparative study of migratory and non-migratory morphotypes of the three-spined stickleback (*Gasterosteus aculeatus* L.) and their

energetics and swimming behaviour  Migrating fish species like the three-spined stickleback show distinct characteristics in their swimming capacities and energetics. Differences between migrating (trachurus-form) and non-migrating (leiurus-form) populations are significant and can be used to estimate the adaptation to migration within one species. Also, comparative studies of freshwater fish migration must consider seasonal and temporal factors which can make results difficult to interpret**.** The three-spined stickleback is a good model for studying migrating and non-migrating types from the same habitat at the same time. In contrast to North American sticklebacks with better endurance capacity in the migratory form and burst capacity in the non-migratory form, the European threespined stickleback of the trachurus type performs better in both, endurance and burst swimming. This shows the European migratory form to be adapted to long diadromous migrations. Migration to spawning grounds requires upstream swimming. It might be assumed that fish migrate at the speed with the lowest cost, at $U_{opt}$. In the present study, $U_{opt}$ differed significantly between the two morphs, with trachurus showing higher values than leiurus. This would indicate that trachurus can swim at faster migration speeds at a relatively lower cost. Swimming at $U_{opt}$, trachurus types consumed a greater amount of oxygen in absolute terms, which would seem unfavourable from a theoretical perspective. Yet the percentage increase in $MO_{2opt}$ compared to the total scope of activity between the two morphs was identical, indicating that the same percent of their aerobic energy budget was directed towards swimming at $U_{opt}$. Also, $CoT_{opt}$ was not significantly different, indicating that when swimming at the same speed, both morphs expended the same amount of energy, and that the elevated $MO_{2opt}$ value was only due to the increased SMR in trachurus

**Chapter Four** presents an integrated study on swimming capacity and energetics in seven European freshwater fish species with different adaptation to migration (brown trout *Salmo trutta fario* and European perch *Perca fluviatilis*, long distance migrating; roach *Rutilus rutilus* and common carp *Cyprinus carpio*, short to middle

range distance migrating; gudgeon *Gobio gobio*, limited migration; bullhead *Cottus gobio* and stone loach *Barbatula barbatula*, very limited to no migration). Critical ($U_{crit}$), optimal ($U_{opt}$) and maximum ($U_{max}$) swimming speed and oxygen consumption ($MO_2$) were analysed. Trout, roach and perch performed excellent, especially the larger individuals, while stone loach and bullhead did not.. This is not surprising since the former are typical long distance migratory species and the lather are not. But also gudgeon, considered to be a non-migrating species, performed surprisingly well. Gudgeon is a bottom dwelling species with limited migration behaviour, therefore we did not expect such high values. However, the scope for activity in this species is low. This suggests that a relatively large part of the $U_{crit}$ can be fuelled by anaerobic metabolism. Therefore fast speeds that have to be maintained for prolonged periods of time might be less advantageous in nature for this fish species.

As swimming performance and energetics vary between fish species, different dia- and potamodromous fish species should be considered when evaluating possible effects of barriers. Therefore, species such as bullhead and stone loach should be taken into account when barriers are remediated by the use of fish passes. Even small obstacles can be a barrier for genetic exchange between populations in short distance migrators

**Chapter Five** discusses biomechanical swimming patterns and manoeuvrability of three different swimming modes. Manoeuvrability of a swimming fish, defined as minimum turning radius per swimming speed, depends on body undulation and flexibility. Anguilliform (eel, *Anguilla anguilla*), subcarangiform (sea bream, *Abramis brama*) and labriform swimming fish (striped surfperch, *Embyotoca lateralis*) show decreasing body flexibility when swimming, using less surface of their body for propulsion. When plotting minimum turning radius against swimming speed, the data points show a random distribution but with a lower limit. The lower limit for turning radius is positively correlated with speed and shows that the sharpness of possible curves of swimming paths is decreasing

with increasing swimming speed. The resulting slope of this lower limit is increasing with decreasing use of the trunk region for swimming. The anguilliform eel, using the largest amount of its trunk region for propulsion, has the lowest lower limit and thus can perform sharp turns even at high swimming speeds, while surfperch, using its pectoral fins for propulsion and not the trunk musculature before gait transition is more limited in the sharpness of turns at higher swimming speeds, which limits its manoeuvrability at high speeds.

In **Chapter Six** I present a study on spontaneous swimming activity in a labriform swimming fish, the striped surfperch (*Embyotoca lateralis*). The objective of this study is to identify associated kinematic variables to quantify oxygen consumption during spontaneous labrifom swimming. Kinematic variables (swimming speed, change of speed, turning angle, turning rate, turning radii and pectoral fin beat frequency) and oxygen consumption ($MO_2$) of spontaneous swimming in *Embiotoca lateralis* were measured in a circular arena respirometer using video tracking. For this labriform swimmer, oxygen uptake did not correlate with speed, acceleration and deceleration, turning angle turning radius but pectoral fin beat frequency ($f_P$) when swimming at low to moderate speeds before gait transition. The results suggest that energy requirements of a labriform swimming fish during spontaneous swimming can be accurately predicted ($r^2$ = 0.71) using the pectoral fin beat frequency. Complementary to other methods within biotelemetry such as EMG it is suggested that such correlations of pectoral fin beat frequency may be used to measure the energy requirements of labriform fish such as *E. lateralis* in the field.

**Chapter Seven** discusses the effect of a chemical barrier on fish swimming and predation. In fish, fast starts are used for escape and predation and are therefore an ecologically important movement. Fast starts are generated by glycolytical muscle performance and are influenced by many internal and external factors. It is known that ammonia pollution has a major effect on the glycolytical muscle action, thus creating conditions in which fast start performance might be

reduced and predation rates altered. Therefore, escape response and predation strikes were investigated in brown trout (*Salmo trutta fario*) exposed to an elevated (1 $mg.1^{-1}$) ammonia concentration. Various locomotor and behavioural variables were measured. After 96 hours of exposure, kinematic factors of escape performance are significantly reduced and the direction of escape is random. Predation strikes are affected and predator behaviour is also altered and the number of prey captured is reduced. The effect of ammonia exposure is more pronounced in large fish than in small fish. This study shows that ammonia exposure affects brown trout escape response mainly through a reduction in fast start velocity and through an impairment of directionality. Thus, in addition to a reduced strength of the response, ammonia exposure may also reduce the fish's elusiveness facing a predator. Predation rate and social interactions are disrupted and predator prey relationships may be altered.

It can be concluded that there are many factors, biotic and abiotic, which influence the capability of fish to clear man made physical or chemical obstacles across migration paths. The present thesis highlights some aspects of swimming physiology and energetics involved in migration of several fish species. Based on the findings of the present work, future studies should involve the measuring of speeds and energy of migrating fish in the field. The actual speed used for migration is unknown, yet. Results on migration speeds could elicit vital information on behaviour and energetics in fish. Also, more relevant tests to measure swimming and migrating behaviour, measured under controlled circumstances, have to be taken into account. $U_{crit}$ tests are too relative when comparing physiological and ecological aspects of the swimming capacity and therefore, new tests for swimming capacities should be developed. More research has to be done on environmental effects on migration, in particular, changes in abiotic factors, such as salinity, pH and temperature, but also in biotic factors, such as species compositions of habitats and diseases. Especially two threats on endangered fish species should be included in this field of study,

which could reveal vital information that can be used to better protect the environment: global warming and bio-invasion. Such studies are vital to investigate and protect migrating fish species and their habitat.

## *General Introduction and Objectives*

Fish species have evolved strategies that enable them to optimise their fitness by using different types of habitats, hence migration between habitats are frequent. However, the term 'migration' also applies to individual fishes since it increases or decreases their fitness (Northcote, 1978, 1998). These movements do not need to involve journeys between biomes to result in a change of the fish's fitness (Lucas and Baras, 2001). As diadromous migrations (McDowall, 1988, 1997) have been the outstanding examples for mobility in fishes and non-diadromous species have been deemed to be resident, they have been largely excluded from management schemes aiming at the restoration of the free circulation of fishes in river systems. Nowadays, there is a bulk of evidence that most so-called resident freshwater fish species undertake potamodromous migrations (for review see Lucas and Baras, 2001). The underestimation of these migrations owes largely to the limited accuracy of the methods employed to investigate them (Gowan et al, 1994).

Spatial mobility in fish serves a series of purposes (ontogenetic, refuge, feeding and spawning migrations). It also enables the recolonisation of habitats where the fish community has suffered catastrophic events of natural (e.g. spates) or anthropogenic origin (e.g. pollution). Free mobility of individual fishes is not only necessary for colonisation processes, but also for maintaining a dynamic equilibrium within the fish community by means of spawning migrations and thus stabilizing populations. Population fragmentation due to habitat fragmentation results in lower effective population sizes, reduced variability and heterozygosity, higher chances for genetic drift and bottlenecking, increased inbreeding and hence reduced fitness, which compromises long-term survival. The exchange of genetic material favours genetic diversity, whereas the reduced exchange of genes leads to speciation or extinction in the long term (Frankham

et al, 2002). Therefore, mobility between habitats is a key component of evolutionary processes that lead to speciation and genetic diversity.

The increase of the human population, the development of agriculture, and the accompanying modifications of the landscape have resulted in a series of impacts on the environment. In order to improve navigation and increase hydraulic control, rivers and streams have been dredged and their banks have been modified, resulting in a loss of essential habitats. Dams and weirs have been erected for flow control, hydro-power and irrigation (Jungwirth et al 1998, 2000; Marmulla, 2001). These obstructions can be impassable and therefore can lead to local extinction of populations, extinction of species and disturbance of communities in the upstream reaches (Lelek, 1987). For example in Belgium, all long-distance migrating fish species have been extinct over the past two centuries in the River Meuse (Phillipart et al, 1988), and in Flanders are only five of the 13 long distance migrating species left, being very rare (Vandelannoote et al, 1998). Weirs and dams, in addition to their direct blocking effect, have a series of side effects, due to modifications of hydrodynamical conditions or other physico-chemical aspects, which may influence fish migration, depending on timing of migration and seasonality. For species with lesser swimming and leaping capacities, small obstacles can have the same impact as dams and weirs, and they also have been the cause, together with habitat loss and fragmentation, of the decline of populations and the threat of extinction of entire fish species (Toepfer et al, 1999; Warren and Pardew, 1998; Warren et al 2000).

Most efforts to solve the problem of habitat fragmentation due to the obstruction of migration routes were put in the introduction of fish passages over huge dams. This resulted in an improvement of upstream movements of migrating fish species, as well as their downstream movements (Clay, 1995; Odeh, 1999; Larinier, 2001, 2002). The adequacy of fish passages depends on their attractiveness to the migrating fish species, minimising time and energy

wasted before migrants enter the fish passage, and on their feasibility, i.e. whether the fish passage can be successfully crossed by targeted species.
Migration, defined as the movement from one habitat to another, is very much depending on swimming behaviour and energetics. The capacity for migration therefore relies on the integration of locomotor activity and associated energy provision. Freshwater fish have a wide range of body forms, energy metabolism and oxygen uptake and transport strategies. The resulting diversity in swimming modes and performances vary from sluggish, serpentine eels and lampreys to the extraordinary acceleration ability in fast starts of esocids, and the impressive sustained swimming performance of diadromous migrators such as salmonids (Webb, 1984, 1994).

**The main objective of this thesis is to increase the knowledge on swimming behaviour, swimming performance and the energetics of fish swimming We studied both migrating and non-migrating fish and related their performance to the capability of migrating and non-migrating fish species to clear man made obstruction on migration paths.**

## ***Outline of the Thesis***

Chapter one gives an introduction to muscle physiology followed by an introduction to fish swimming and finally to the associated costs and energetics.
Chapter two consists out of a more technical paper, answering the question if flume length and fish size have an effect on performance in an $U_{crit}$ test. Since we studied fish of different sizes it was important to asses possible influences of the type and size of swimming flume on the results obtained. These results are presently *in press* in the *Journal of Fish Biology* (Longer flumes increase critical swimming speeds by increasing burst and glide swimming duration in carp (*Cyprinus carpio*, L.) by C. Tudorache, P. Viaenen, R. Blust, G. De Boeck).
In chapter three the question will be raised to what extent different fish species representing different migratory strategies, show adaptations of their energy metabolism and swimming capacities to migration and to which extent they are able to clear man made obstructions across their migration paths. We present an integrated study on swimming capacity and energetics in seven European freshwater fish species: brown trout *Salmo trutta fario* and European perch *Perca fluviatilis* (long distance migrating); roach *Rutilus rutilus* and common carp *Cyprinus carpio* (short to middle range distance migrating); gudgeon *Gobio gobio* (limited migration); bullhead *Cottus gobio* and stone loach *Barbatula barbatula* (very limited to no migration). These results are at present *in press* in *Ecology of Freshwater Fish* (A comparison of seven European freshwater fish species in terms of swimming capacity and energetics: adaptations to migration. Tudorache, C, P. Viaenen, Blust, R and De Boeck, G.).
Are migrating and non-migrating morphs of three-spined sticklebacks (*Gasterosteus aculeatus* L.) different in terms of swimming capacity and energetics? That is the question answered in chapter 4. This paper appeared in *Journal of Fish Biology* (Swimming capacity and energetics of migrating and non-migrating

morphs of three-spined stickleback (*Gasterosteus aculeatus* L.) and their ecological implications. by C. Tudorache, R. Blust and G. De Boeck).

Anguilliform (eel, *Anguilla anguilla*), subcarangiform (sea bream, *Abramis brama*) and labriform swimming fish (striped surfperch, *Embyotoca lateralis*) show decreasing body flexibility when swimming, using less surface of their body for propulsion. Chapter 5 describes whether different kinds of swimming morphologies in fish show a possible adaptation to manoeuvrability. The manuscript resulting from this work will be submitted to *Journal of Experimental Biology* (The Amount of Trunk Undulation Increases Turning Radii with Increasing Speed by Tudorache, C., Jordan, A. D., Domenici, P.)

In chapter 6, examining the energetics of free swimming in a labriform swimming perciform, the following question will be answered: 'Is there a predictor of oxygen consumption that can be used in the wild?' The resulting paper has been submitted to *Copiea* (Pectoral fin beat frequency predicts oxygen consumption during spontaneous activity in a labriform swimming fish (*Embiotoca lateralis*). C. Tudorache, A.D. Jordan, J.C. Svendsen, P. Domenici, G. De Boeck and J.F. Steffensen)

In chapter 7, we studied the impact of ammonia exposure on the fast start and prey capture ability of brown trout. The effect of exposure on migration and the ecological relevance of such pollution are examined. The resulting paper has been submitted to *Journal of Experimental Biology* (Ammonia exposure affects fast start performance and predation behaviour in brown trout (*Salmo trutta fario*, L.) by C. Tudorache, R. Blust, G. De Boeck).

Finally, chapter 8 gives the general conclusions and recommendations for future research.

***Chapter 1***

# *Introduction to Muscle Physiology, Fish Swimming and Swimming Energetics*

### ***Muscle Physiology***

Fish lateral muscle fibres have more than one nucleus and they are surrounded by a plasma membrane (sarcolemma). They are packed between sheets of collagen (myosepts) into cone shaped myotomes which are stacked in a metamerical arrangement on both sides of the median septum. The muscle fibres' striated appearance is due to the regular arrangements of thick and thin filaments in bundles (myofibrils). In the longitudinal direction, myofibrils consist of a sequence of identical units (sarcomeres), interconnected by Z-lines, disclike structures. The sarcomeres contain two kinds of filaments, causing muscle contractions: a) Thin filaments, attached to the Z-lines being shorter than the sarcomere length, and b) thick filaments, running parallel to the thin ones, being shorter than the sarcomere length. Successive sarcomeres contain thin filaments (I-bands) and thick filaments (A-bands), and M-lines, marking the middle of the sarcomeres. During contraction, thin filaments with actine molecules slide along thick filaments with myosine as active molecule. Actine and myosine form cross bridges cyclically between each other and generate forces dragging thin filaments along thick ones; the sarcomere is shortened and creates tension. ATP is converted into ADP by a sudden occurrence of high Ca-concentration. The breakdown of ATP provides the energy needed for the build up of tension. For immediate use, the ATP concentrations are maintained by stores of phosphocreatine splitting into phosphate which is added to ADP to create ATP and creatine. (Eckert et al, 2001; Videler, 1993; fig. 1).

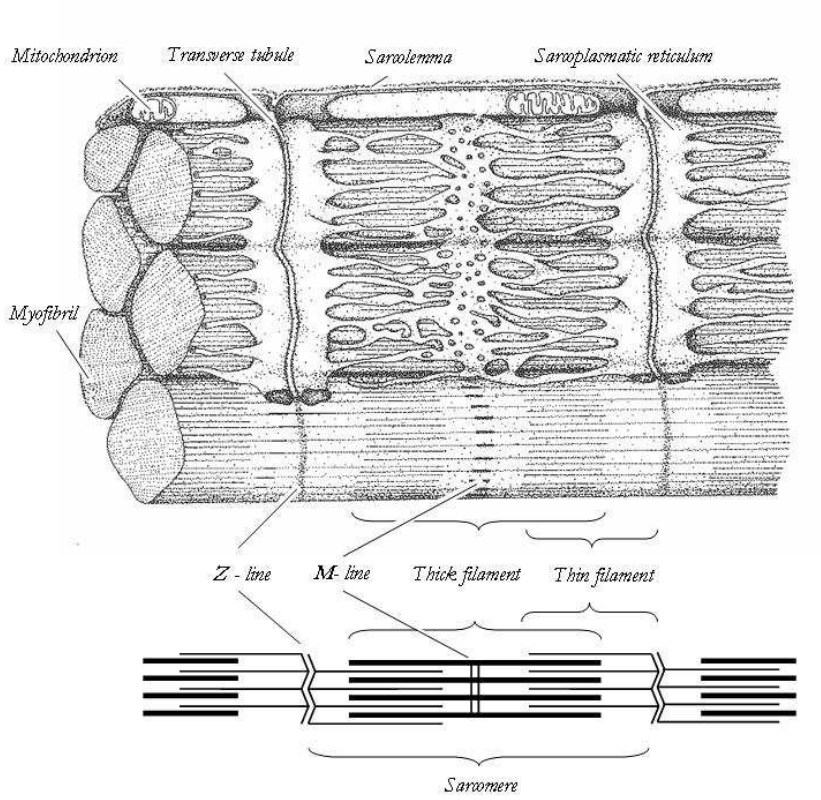


Fig. 1: Diagrammatic presentation of the structure and nomenclature of a vertebrate muscle fibre. The fibre is surrounded by the sarcolemma, contains mitochondria and is densely packed with myofibrils. Each myofibril is in close contact with the sarcoplasmatic reticulum and consist of a series of sarcomeres. The overlap between thick and thin muscles increases when the muscle shortens curing contraction, decraseing the distance between the Z-lines. A transverse tubule system, open to the outside through holes in the sarcolemma coincides with the positionb of the Z-lines. The diagram is wredrawn after Penzlin (1989).

A system of branchial tubules, the T-tubules, appearing at regular distances along the fibres, connects the outer with the inner milieu through holes in the sarcolemma and conduct electrical stimuli into the fibres. The sarcoplasmatic reticulum is surrounding the myofibrils. The lumen of these tubules is connected with the T-tubules by so-called terminal cysternae. To relax the muscle, the sarcoplasmatic reticulum decreases the calcium concentration around the myofibrils. To activate the myofibrils the T-tubules release calcium when triggered by neuromotoric signals. Therefore, the speed of activation depends on the intensity of the contact between T-tubules and sarcoplasmatic reticulum (Akster, 1981, 1985 in Videler 1993). Situated between the sarcolemma and the

myofibrils, mitochondria produce ATP by aerobic phosphorylation, using the oxidation of fat, protein or glycogen as energy source (van den Thillart, 1986).

When dissecting a fish body, the muscles appear in two different colours: red and white. In some species this colour difference is very obvious but in many others the arrangement is more diffuse. Also pink muscles are known. The proportion of the three differently coloured fibre types is related to life style (Boddeke et al, 1959 in Videler, 1993). Pelagic fish species have a high proportion of red fibres while fish species that spend most of their time lying on the bottom have muscle fibres that are virtually all white. Also, the relative cross sectional area occupied by red or white muscle fibres changes along the fish body from head to tail.

The biggest portion of fish muscles is white. They are larger than red muscles and the white colour is caused by lesser vascularisation and a lack of myoglobine. These fibres are fast twitch, that is, they contract at a high speed, producing high contraction forces, but they become exhausted quickly. White muscle fibres contain few mitochondria and mainly produce ATP by hydrolysis of stored phosphocreatine to maintain the concentration. Subsequently, glycogen is turned into lactic acid by means of anaerobic pathways. This supplies energy more rapidly, but it has disadvantages, too. Firstly, the net ATP production per mole glucose is only a fraction of that produced through the aerobic pathway and. Secondly, lactic acid has to be excreted and oxidised or has to be oxidised in situ before the muscle fibre can be active again. The removal of the lactic acid produced by the anaerobic processes can take up to 24 hours after an all-out burst of activity.

Red muscle fibres, being generally much thinner than white fibres, are about 20 to 50% of their diameter. Red muscles contract slowly but they are virtually inexhaustible due to their aerobical metabolism, producing energy in the mitochondria. The red colour is due to the extensive blood supply and presence

of myoglobine. The muscle fibres contain high amounts of large mitochondria and the activity of oxidative enzymes is higher than in white muscles.

Pink fibres, intermediately situated as a distinct layer between red and white fibres, produce energy through aerobic metabolic processes, showing generally intermediate characteristics between white and red muscle fibres. In salmonids, for example, the white muscle layer is pink and consists of large diameter fast and small muscle fibres being structurally different from red fibres.

## ***Swimming***

*- Steady swimming -*

According to Breder (1926) there are different combinations to move the body and fins for swimming among fishes. A swimming fish can use several gaits involving different propulsors. Generally, fishes either use median and paired fins (MPF) or the body and caudal fin (BCF) for swimming (Fig. 2, 3).

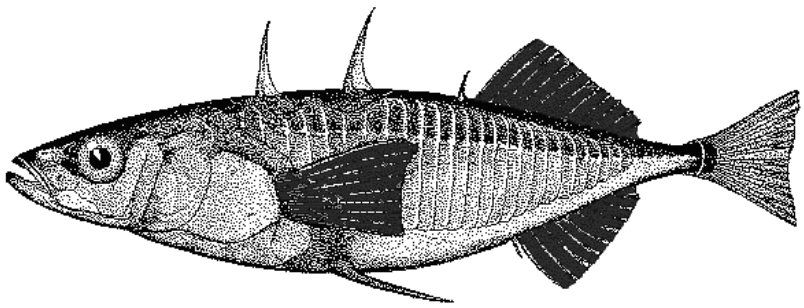

Figure 2: The Stickleback (G*asterosteus acculeatus*, L.) is a typical MPF (Median Paired Fin) swimmer, using median and paired fins (shaded) for propulsion at low swimming speeds.

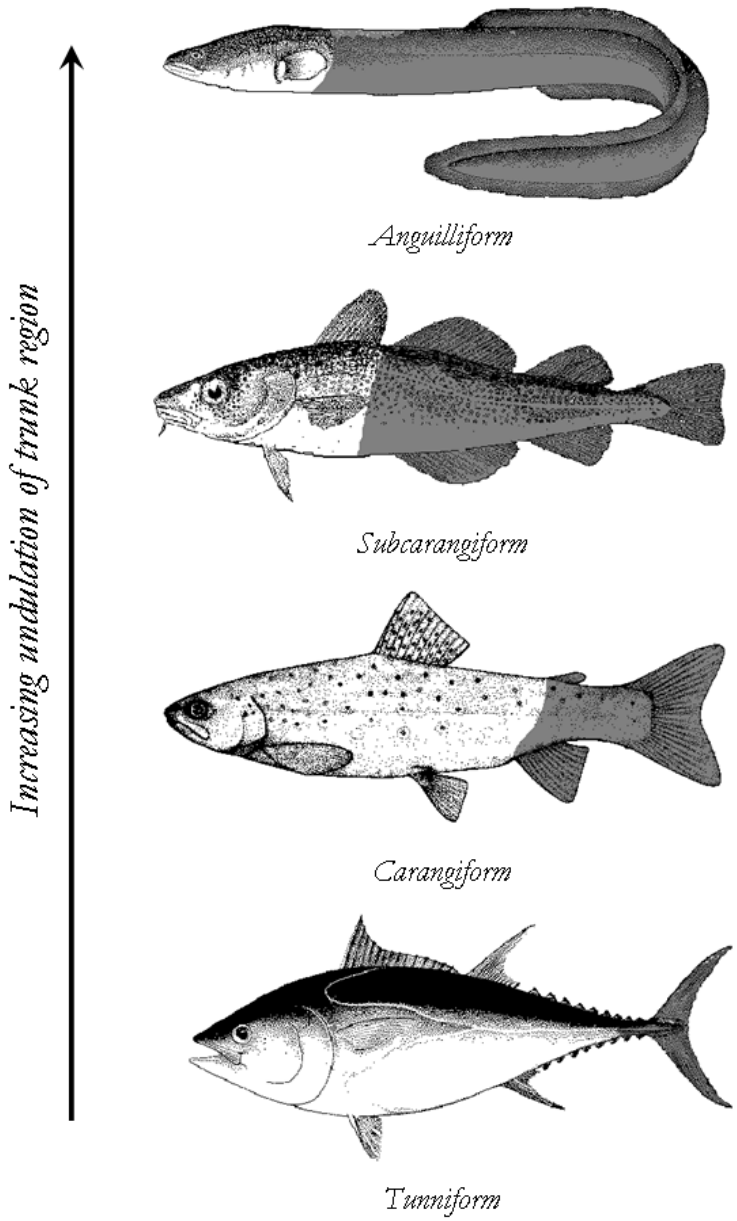


Figure 3: BCF (Body Caudal Fin) swimmers showing increasing undulation of the trunk (shaded) while swimming. Each swimming mode is represented by a species: Angulliform – European eel (*Anguilla, Anguilla* L.), Subcarangiform – Atlantic cod (*Gadus morhua* L.), Carangiform – Brown trout (*Salmo trutta fasrio* L.), Tunniform – Bluefin tuna (*Thunnus thynnus* L.)

MPF are used at low speeds, as they use only a small amount of muscles. Here, the efficiency can be higher compared with BCF (Webb 1993). BCF provide a greater power output and therefore are used at high speeds and accelerations (Webb 1984). MPF swimming fishes therefore often tend to change gait and use BCF at high speeds, thus increasing their performance. For BCF swimmers,

performance is dependent on body undulation during swimming with low body undulation being an adaptation to high performance as a rigid body reduces drag force (tunniform swimming). (Fig. 3). Most fishes, however, are designated subcarangiform swimmers (Breder, 1926), i.e. they use body undulation for propulsion. Frequency of thrust producing fin beats will increase almost linearly with increasing swimming speeds (Hunter and Zweifel 1971, Wardle et al. 1989, Scharold et al. 1989). Amplitude also will increase linearly with speed until a plateau is reached (Bainbridge 1958, Webber et al. 2001).

*- Unsteady swimming -*

Swimming at uniform velocities along a straight path is rather uncommon among fish. Unsteady movements are common in the behavioural repertoire of most fish. The kinemtaics of fast start, rapid turns, breaking and burst and coast swimming are of great biological relevance.

Unsteadyness is experessed as the rates of change of speed, i.e. acceleration and decelerations. This can be calculated directly by double differentiation of displacement of the centre of bodymass (CoM) as a function of time. The displacements have to be accurately measured from high speed film or video frames. Below, a few unsteady swimming modes, measured in this study are introduced.

*- Burst-and-coast swimming -*

Burst-and-coast (or burst-and-glide) swimming behaviour is commonly used by several species. It consists of cyclic bursts of swimming movements followed by a coast phase in which the body is kept motionless and straight. The burst phase starts off at an initial velocity ($U_i$), lower than the average velocity ($U_c$). During a burst the fish accelerates to final velocity ($U_f$), higher than $U_c$. The cycle is completed when velocity $U_i$ is reached at the end at the deceleration during the coast phase (Videler and Weihs, 1982). In a swimming tunnel, the start of a burst

is defined in terms of: 1) a large and discrete increase in upstream motion, 2) increased tail span and 3) decreased tail-beat period.

Figure 4 shows burst-and-coast swimming of a carp (*Cyprinus carpio* L.) in a swimming tunnel of 3 meters with limited space to swim freely. As the fish is forced to swim against a constant water stream, $U_i$ is measured as a ground speed in cm $s^{-1}$ (i.e. the speed of the swimming fish according to the ground) at the start of the burst and $U_f$ is the maximum speed reached in the tunnel.

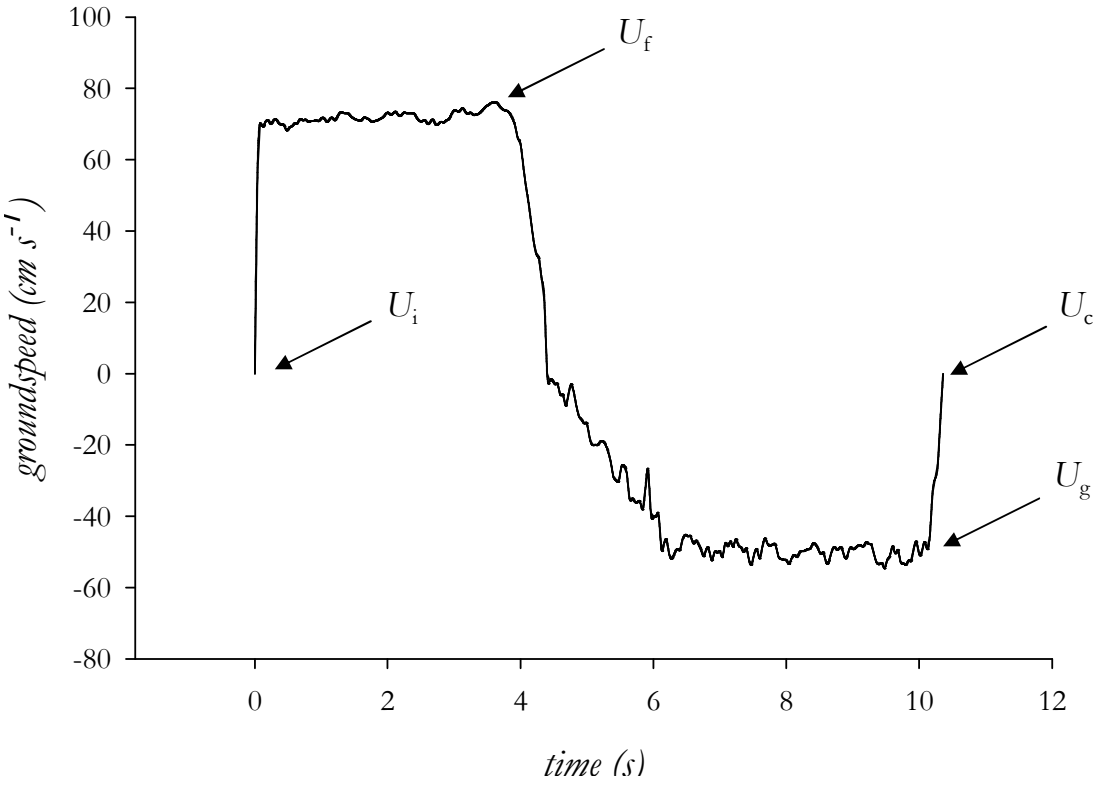


Figure 4: An example of a bursting and gliding carp in a swimming tunnel of three meters. See text for details.

The gliding phase results in a backwards movement as the fish is swept downstream and the resulting ground speed is negative ($U_g$). As the fish is then starting to swim actively again in order not to touch the grid installed at the downstream end of the swimming tunnel, the ground speed reaches zero, being an equivalent with $U_c$, which in this case is the water speed and thus zero ground speed (for a detailed description of design and function of swimming tunnel, see below). Burst-and-coast swimming costs 50% less energy than steady swimming

at the same average speed during low (Weihs, 1974) and high swimming velocitites (Videler, 1981). These model predications are based on a substantial difference in drag between a rigid body and an actively moving fish.

*- Fast starts -*

Fast starts, highly energetic swimming bursts, started either from rest or imposed upon periods of steady swimming (Jayne and Lauder, 1993, Domenici and Batty, 1994), are ecologically important movement patterns in fish. They are used for escaping predators or for achieving prey capture. In some cases they serve for communication between individuals (Fernald, 1975). Kinematically, two different types of fast starts can be distinguished: 1) C-start and 2) S-starts.

1) C-starts are generally used for escape responses. This type of fast start, where a fish takes in a C-like body shape before bursting, results from a contraction of the lateral musculature on the opposite side relative to the stimulus. It can be divided into three kinematic stages: stage 1, a preparatory stroke, stage 2, a propulsive stroke and stage 3, a variable stage involving continuous swimming or coasting (Weihs, 1973). Stage 2 can also be described as the change in the turning direction of the anterior body midline (Domenici and Blake, 1991, 1993). When in stage 2 a contralateral stroke occurs, generated by a contarlateral muscular contraction, the C-start is called 'double-bended'. A 'single-bended' C-start lacks a cortalateral stroke. The distinction between these two types depends on the purpose of the study. C-starts are used only for escape responses (Domenici, 1997) as centre of mass (CoM) of the escaping fish can be displaced using small turning angles, and advantage to escaping the attacking predator (fig. 5).

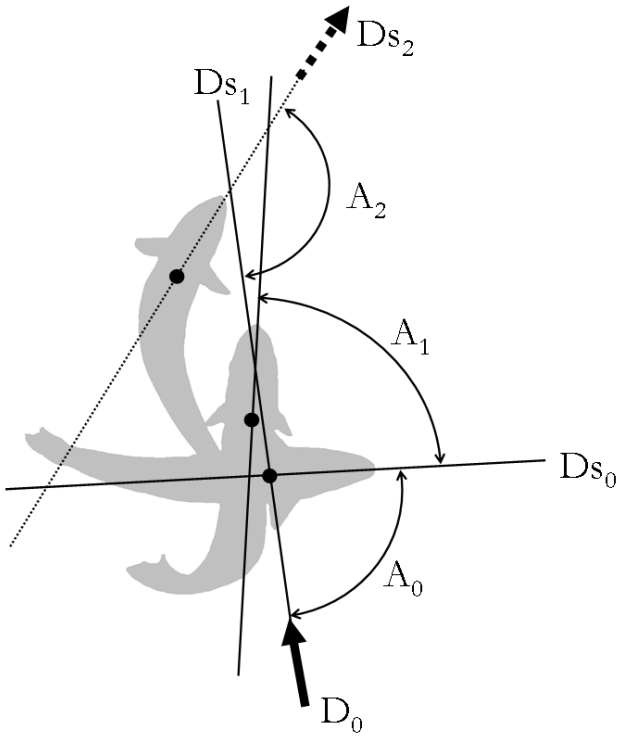


Figure 5: An example for a C-start in a brown trout (20cm body length) when startled as an illustration of the angular variables (solid arrow: stimulus direction; dotted arrow: escape direction). $D_0$, line passing through the stimulus position and the fish centre of mass (CoM); $D_{S0}$, line passing through the CoM and the head of the fish at the onset of the escape response; $D_{S1}$, line passing through the CoM and the head of the fish at the end of the stage 1; $D_{S2}$, line passing through the CoM and the head of the fish at the end of the stage 2; $A_0$, initial orientation; $A_1$, stage 1 angle; $A_2$, escape trajectory

These fast starts are generated by the Mauthner-system, a neurological system that transforms sensory inputs into contralateral muscle contraction and is directed via pair wise situated Mauthner cells in the spinal ganglia. This pair of giant medullary neurons project directly to spinal motor neurons. This circuit is optimal for fast responses by two distinctive features: a) the small number of synapses intervening between the receptor cells and the motor neurons and b) the large size of the Mauthner axons; these axons are about 50 µm in diameter in a goldfish. The appropriate direction of escape originates from two features: 1) each Mauthner cell projects only to contralateral motor neurons and 2) a local network of bilaterally projecting interneurons inhibits activity in the Mauthner

cell away from the side on which the vestibular activity originates. Thereby, the Mauthner cell on the appropriate side generates action potentials leading to contractions of contralateral trunk musculature and moving the fish out of the path of the oncoming predator Conversely, the Mauthner cell on the opposite side is silenced by the local inhibitory network during the response (Purves *et al.*, 2001).

2) With S-starts an S-like shape can be observed, resulting from simultaneous contraction from both sides of the musculature. This fast start pattern is also used for escape but mainly for predation (Domenici and Blake, 1997). The general movement pattern of an S-start is more linear but maximum velocities and accelerations are slower than in C-starts. Here also, enervation of lateral muscles occurs via Mauthner cells (Domenici and Blake, 1997).

For obvious reasons, fast starts are ecologically relevant. Predator prey relationships are shown to be influenced by kinematics. Walker and co-workers (2005) have shown that faster escape starts increase the chance of the prey to evade predators. Dill (1974) demonstrated that the prey's reactive distance increases with speed and depth of the predator's body profile. A rapidly approaching predator may trigger an early response in the prey. Therefore, fast start speed of attacking predators is often sub-maximal (Webb, 1984, Harper and Blake, 1991). Also, predators aim at the centre of mass of their prey, perhaps because the centre of mass being the best target for a predator, since it can be located from the prey's geometry and it also is the point of a prey that moves the least during escapes (Webb and Skadsen, 1980). Domenici and Blake (1991) and Eaton and Emberley (1991) showed that turning angles in escape responses are variable but mostly away from the attacking predator (Domenici and Blake, 1997). Also striking angles of the predator may be species specific (Hoogland *et al.*, 1956) and are possibly influenced by gape limitation in addition to prey shape

and other morphological factors, such as false eye spots (Domenici and Blake, 1997).

*- Rapid Turns -*

In the movement patterns of fish, quick tight turns can be used for various purposes, ranging from escaping predators over social interactions, such as mating behaviour, to manoeuvring through a dense habitat and feeding. Turning radii, i.e. the radii of a circle that can be described by three consecutive points, are a good indicator of the 'tightness' of turns. According to Videler (1993) the minimum turning radius of a fish depends on body flexibility, the degree of lateral compression and the lateral area to generate thrust. Also, the radius is independent of speed and acceleration.

## *Swimming Costs*

*- General Energetics -*

Energy budgets can be described by means of energetic inputs and outputs of an animal, because energy can be converted but never destroyed, according to the thermodynamical law. Bio-energetics (i.e. energy utilization) describes the nutritional energy within an organism. Therefore, nutritional energy may either be partitioned into growth or accumulation in energy stores, be excreted, or support metabolism and activity.

As a fish consumes food, its energy (*C*) is used for production (*P*), respiration (*R*), or is excreted as waste products (*E*), stated in the equation:

$$C = P + R + E$$

Each of these terms in the energy budget are expressed in joule (*J*, Calow, 1985). Some of this energy is covering the costs of the standard metabolic rate (SMR). A larger fraction is required to fuel the active metabolism including the locomotor activities, referred to as active metabolic rate (AMR). All fractions of respiration are depending on factors such as temperature and swimming speed.

*- Estimation of Swimming Costs: The Hardware -*

Swimming costs include all energy expenditure needed to generate movements and forces to interact with the water (Videler, 1993). Fish use oxygen ($O_2$) to burn energy rich substrates obtained from food (proteins, lipids and carbohydrates). For swimming, oxidation produces energy required to make ATP, which fueles the muscles to generate force. Byproducts are carbondioxyde ($CO_2$), water and heat. Thus, fuel depletion rate, oxygen consumption rate, $CO_2$ production rate or heat production can be used to estimate energy turnover during swimming. $O_2$ consumption rate has been used most commonly to estimate the costs of swimming. Therefore, swimming respirometers have been introduced to measure aquatic performance (Fry & Hart, 1948). There are a number of water tunnel respirometer types (Cech, 1990; Lucas et al., 1993), but the most widely used configurations are patterned after two designs: 1) Brett's (1964) annular tunnel and 2) the Blazka et al.'s (1960) coaxial circuit.

1) In order to allow fish to swim at certain controlled speeds, Brett (1964) introduced a swim tunnel with a circular water flow. This type of swim tunnel generally consists of a circular tube that is connected to a pump creating a controllable water flow inside the tube. The swimming section, i.e. the part of the tube into which the fish is introduced, is closed from the rest of the tube by means of a grid or net. To stabilise the water flow and to make it more laminar, so-called honey combs, i.e. small, parallel organised tubes, regulate the flow. Also, Brett-type tunnels usually have better controlled, more uniform flow conditions since they use low-angle bends and an elongated upstream return section to minimize turbulence and vortices.

A great disadvantage of Brett-type swimming tunnels is the large volume they require to create a highly linear flow. When measuring oxygen concentrations in

the water in order to study the oxygen consumption of a swimming fish, a low water/fish volume ratio is advantageous (Steffensen, 1984).

2) Blazka et al (1960) designed a swimming tunnel that consist of concentric tubes and therefore can work without an extended return circuit. The inner tunnel, the actual swimming chamber, lies within an outer tube and the water flowing through the swimming chamber returns through the space between the outer and the inner tunnel. The flow is created by means of a centrifugal pump, i.e., a pump which creates a pressure that presses the water in perpendicular direction of the rotation axis of the screw. Linearity of the flow is maintained by means of a honey comb.
The Blazka design has inherently more flow irregularity but can have a much lower volume, a great advantage in respirometric studies when studying small fishes or those having low metabolic rates (Steffensen, 1984)

*- Estimation of Swimming Costs: The Software -*

Generally, the methodology of $O_2$ consumption, measured in a respirometer, consists of introducing the fish in the swimming chamber, sealing the chamber, so that no air can enter and disturb the accuracy of the measurements and then driving up the water speed inside the swimming tunnel. Doing this, the fish has to swim against this controlled water speed in order to hold its position. Thereby it swims with the same speed as the water flows and the oxygen consumption of the fish can be measured. When plotting metabolic rate against swimming speed in fish, the resulting curve can be described by the exponential equation

$$MO_2 = SMR \cdot e^{c \cdot U}$$

or by the polynomial equation

$$MO_2 = SMR + bU^c$$

with $MO_2$ being the specific oxygen consumption in μmol $g^{-1}$ $h^{-1}$, *SMR* (standard metabolic rate) being the metabolic rate at rest, i.e., as extrapolated to zero activity and *b* and *c* being constants.

The standard metabolic rate (*SMR*) includes also the extra energy needed to bring the organism to an increased activity level but does not comprise the energy needed to swim at a particular speed. The total energy used during swimming is commonly referred to as the active metabolic rate (*AMR*). The difference between *SMR* and *AMR* is called the *scope for activity* and gives an indication of the amount of energy available for locomotion. The levels of these separate metabolic rates are depended on species, size, temperature and velocity. Condition and training also affect the rates.

As lower the metabolic costs of a given swimming speed as higher is the endurance. The swimming speeds increase with decreasing endurance. Swimming speeds can be described in terms of sustainability and duration. The terminology distinguishes several categories of swimming speeds according to duration and function.

1) Sustained swimming speeds can be maintained for long periods without resulting in muscular fatigue. For practical purposes sustained swimming speed is defined as the swimming speed that can be maintained for 200 minutes or longer. Metabolism during sustained swimming speed is aerobic without accumulation of lactate (Jobling 1995). One type of sustained swimming speed can be found at the 'optimal swimming speed' ($U_{opt}$) where the tangent from the origin touches the curve (fig. 5) and describes the speed at which aerobic costs are the lowest (Tucker, 1970), i.e., where the amount of work per meter swum (*WMP*) reaches a minimum. The $U_{opt}$ can also be found by solving the exponential function

$$MO_2 = SMR \cdot e^{c \cdot U}$$

for *c*, because when using the exponential metabolic function only *c* will affect $U_{opt}$ and the resulting formula

$$U_{opt} = \frac{1}{c}$$

gives the optimal swimming speed (Petterson & Hedenström, 2000).

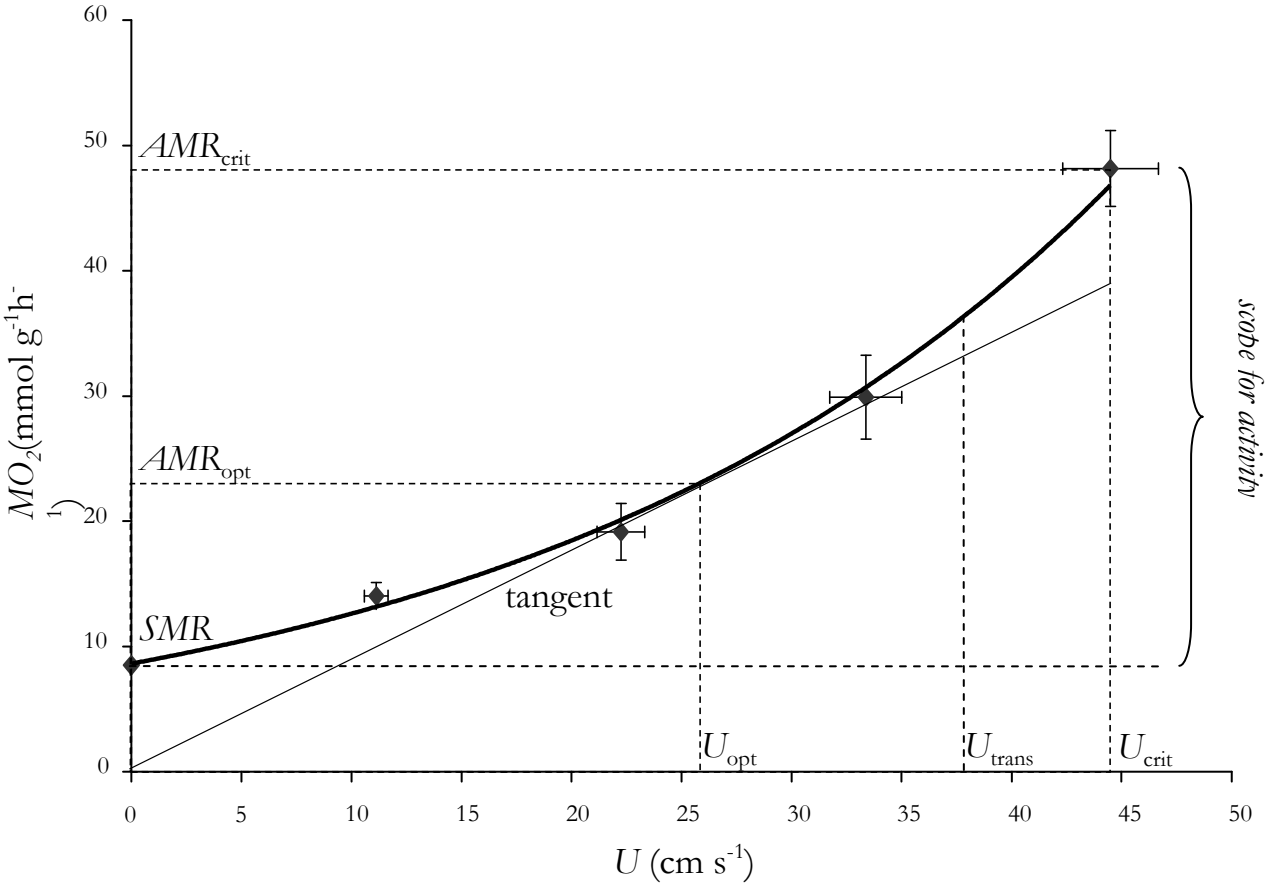


Figure 5: A typical swimming curve of Stickleback (*Gasterosteus aculeatus L.*), swimming at arrange of speeds in a swimming respirometer. $MO_2$ (mmol $g^{-1}h^{-1}$), i.e. the amount of $O_2$ consumed per time unit and body weight is plotted against swimming speed ($U$). The resulting curve is extrapolated to zero activity at the *SMR* and different speeds ($U_{opt}$, $U_{trans}$, $U_{crit}$) and energetic levels ($AMR_{opt}$, $AMR_{crit}$) can be calculated. For explanation see text.

In order to compare optimal swimming speeds over different conditions from the polynomial equation

$$MO_2 = SMR + bU^c$$

the *WMP* can be found by definition

$$WMP = AMR\ U^{-1} = SMR\ U^{-1} + b\ U^{(c-1)}\ (\mathrm{Jm^{-1}}).$$

Differentiating with respect to $U$ results in

$$WPM' = -SMR\ U^{-2} + (c-1)bU^{(c-2)}$$

which is zero when

$$U_{opt} = \{SMR/[(c\text{-}1)b]\}^{1/x} \text{ (m s}^{-1}\text{)}$$

When dividing by body weight to obtain the dimensionless cost of transport (CoT), the minimum amount of energy required per unit weight and per unit distance ($JN^{-1}m^{-1}$ or -), is given by:

$$CoT = AMR_{opt} (M\, g\, U_{opt})^{-1}$$

with $AMR_{opt}$ being the active metabolic rate at $U_{opt}$, $g$ the acceleration of gravity in $ms^{-2}$ and $M$ the body mass in kg. With the help of this dimensionless number, comparisons can be made easily. $U_{opt}$ in $ms^{-1}$ is positively correlated with mass. $U_{opt}$ decreases with mass if it is expressed in $blm^{-1}$. $CoT$ is negatively correlated with mass.

2) Prolonged swimming speeds are of shorter sustainability (20 - 200 min) and energy supply may be provided from both, aerobic and anaerobic metabolism. A special category of sustained swimming is the critical swimming speed ($U_{crit}$) that is determined under controlled laboratory conditions in swimming tunnels. This swimming speed is used to compare experimental factors and conditions. The $U_{crit}$ is defined as the nominal speed at which maximum oxygen uptake occurs (Webb 1971; Farrell & Steffensen 1987) at the $AMR_{crit}$ (Active Metabolic Rate at $U_{crit}$; fig. 5). As in most cases burst and glide swimming is applied at $U_{crit}$, the metabolism is both aerobic and aerobic. The determination of $U_{crit}$ occurs according to the equation

$$U_{crit} = U_i + [\Delta U(T_i\, \Delta T^{-1})]$$

with $U_i$ being the highest velocity maintained for the whole time interval, $\Delta U$ the velocity increment (here: 5 cm $s^{-1}$), $T_i$ the time elapsed at final, fatigue velocity, and $\Delta T$ the time interval (Brett, 1964). The fish is assumed to be fatigued after touching the down stream grid of the swimming tunnel three times.

The change from aerobically to anaerobically fuelled swimming is termed gait transition ($U_{trans}$). This is the transition from sustained swimming gait to burst

and glide gait. The transition is not a fixed speed but rather a period of speeds where both gaits are applied simultaneously. Therefore, this swimming speed is determined in a swimming tunnel as the speed at which three consecutive changes from steady to burst and glide mode occur (Fig. 5)

.

***Chapter 2***

# *Longer Flumes Increase Critical Swimming Speeds by Increasing Burst and Glide Swimming Duration in Carp (Cyprinus carpio, L.).*

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***Abstract***

Carp (*Cyprinus carpio, L.*) alters the repertoire of swimming behaviour with increased flume length. While the transition speed from steady to burst-and-coast swimming was unaffected by flume length, fish reached higher $U_{crit,}$ consequently swimming for longer periods of time in burst-and-coast mode and hence performing more work before becoming fatigued. Analysis of swimming behaviour of burst-and-coast swimming revealed an increase in duration and a decrease in distance of forward burst movements with increasing water speeds. Frequency was unaffected by water speed. Overall longer flumes increase $U_{crit}$ by allowing for less restricted burst-and-coast swimming behaviour.

## *Introduction*

Migrating fish swim against currents that vary in water speed. In the laboratory, swimming ability is commonly tested by means of increasing velocity tests as measures of the ability of fishes to transit these variable currents. Increasing velocity tests determine the critical swimming speed ($U_{crit}$; Brett, 1964), the highest maintainable swimming speed for a period equal to the time interval used in the test (Peake *et al.*, 1997). Time intervals vary (Beamish, 1978) from over an hour, covering speeds at which it is generally assumed that maximum oxygen uptake occurs (Webb, 1975; Farrell & Steffensen, 1987; Keen & Farrell, 1994; Gregory & Wood, 1998), to 1 or 2 minutes, which approach sprint speeds. In all situations, prolonged performance is examined where metabolic energy is derived from both aerobic and anaerobic pathways and fatigue ultimately occurs. Determination of $U_{crit}$ is a good indicator for the capacity of an upstream migrating fish to swim through strong currents, and also can be used as a measure for the impact of environmental factors such as temperature, hypoxia, diseases or toxicants (Brett & Glass, 1973; Waiwood & Beamish; 1978, Beamish, 1978; Thomas & Rice, 1987; Nikl & Farrell, 1993; Hammer, 1995).

Critical swimming speeds are measured in flumes, which are well known to restrict behaviour to rectilinear swimming. In addition, flume design, specifically chamber length potentially affects gait expression, by restricting behaviours based on moving forwards and back in a current. Thus, as fish approach $U_{crit}$, they tend to shift from a steady swimming gait to an unsteady burst-and-coast gait, involving a rapid upstream burst followed by coasting back downstream.

Burst-and-coast swimming is an energy-saving behaviour increasing endurance at prolonged speeds. Therefore, the ability to fully exploit the burst-and-coast gait would be expected to affect maximum speeds attained before fatiguing. Haro *et al.* (2004) showed that fish in a long (24 m) raceway reached higher swimming speeds than in a shorter flume, a result supported by studies by Peake & Farrell (2006) and Castro-Santos (2004, 2005).

This study seeks to determine how flume length affects the duration and composition of the unsteady gait leading up to fatigue at $U_{crit}$. It was hypothesised that carp (*Cyprinus carpio, L.*) would reach higher $U_{crit}$ in a long flume than in a short one due to a greater ability to express the burst-and-coast gait.

## *Materials and Methods*

### *- Stock -*

Four sizes classed of carp (1-4) with body lengths ($L_B$) of 5.31 ± 0.35, 10.04 ± 0.20, 19.85 ± 0.26 and 26.21 ± 2.49 cm, respectively, (mean ± SD) were held at the Hydrological Institute in Antwerp in 300 litre tanks fed with 100 litres per day of charcoal filtered tap water of 22 ± 2°C for three weeks before experiments started. Fish were fed with 'Pond Sticks' (Tetrapond, Henckel, Germany) three times a week at a 2 % body mass ratio.

### *- Determination of $U_{crit}$ -*

Eight fish per size class were tested individually in a Brett-style swimming tunnel with a cross section of 0.4 x 0.4 m and a total of length 20 m. The total circulating volume of filtered freshwater was 8000 l. Fish swam in the downstream 3 m of the observation section of the flume, either having access to the entire length (long flume) or being restricted to a shorter portion using a metal grid (short flume). The short-flume lengths were scaled to fish length and so were 35 cm for size classes 1 and 2, 70 cm for size class 3, and 87 cm for size class 4.

For both long and short flumes, fish were provided with a 2-hr acclimation period swimming at 5 cm $s^{-1}$. After this period, water velocity was increased in increments of 5 cm $s^{-1}$ at intervals of 20 minutes, until fish fatigued. Velocity was controlled by a computer. Fish were first swum in the long flume, after which

the grid reducing the length was inserted. After a further 2 hour acclimation period, the increasing velocity test was repeated.

Fatigue was determined using a photoreceptor-based automated system measuring fish contact with a metal grid delineating the downstream end of the flume. Fatigue was defined as occurring when a fish touched the grid three times in three seconds or longer. $U_{crit}$ was calculated according to the equation

$$U_{crit}=U_i+[\Delta U(T_i\,\Delta T^{-1})] \ ,$$

with $U_i$ the highest velocity maintained for the whole time interval, $\Delta U$ the velocity increment (here: 5 cm $s^{-1}$), $T_i$ the time elapsed at final, fatigue velocity, and $\Delta T$ the time interval (here: 20 min; Brett, 1964). After $U_{crit}$ measurements were finished, fish were removed and the body mass length, height and width measured.

*- Swimming behaviour -*

Two aspects of swimming behaviour were recorded, the nature of bursts and coasts that make up burst-and-coast behaviour and the time over which this gait was used. The start of a burst was defined in terms of: 1) a large and discrete increase in upstream motion (see Figure 2), 2) increased tail span and 3) decreased tail-beat period. The transition to the burst-and-coast gait was defined as the speed at which the first three burst-and-coast sequences occurred.

*- Changes with current speed -*

While fish of size classes 2, 3 and 4 were swimming in the long flume (3 m), a digital PAL video camera (Sony Corporation DCR-HC39E), positioned at the side of the swimming flume, was used to film behaviour. Films in AVI format were imported into Vernier Logger Pro 3.3 and the XY coordinates of the tip of the head were determined. Velocity, frequency, distance and duration of bursts were established by determining the position of the tip of the head in each frame over time. Average and maximum speed were averaged for three randomly

chosen bursts at each speed up to $U_{crit}$. Statistical analysis was performed by One Way ANOVA (GraphPad InStat version 3.05). When $P<0.05$, the Tukey test was used as posthoc test. Results are given as mean ± standard deviations.

## ***Results***

*- Swimming behaviour -*

Fish swam steadily up to gait transition speeds of 38 cm $s^{-1}$ for the smallest size class, and speeds of 45 cm $s^{-1}$ for larger size classes (Figure 1). Differences in gait transition speeds measured in the long and short chambers were not significant (One-way ANOVA, $p>0.05$), but did increase significantly with fish length (linear regression, $p<0.001$). Burst-and-coast behaviour continued until fish fatigued at $U_{crit}$. Bursts were clearly delineated from steady swimming by a rapid acceleration (Figure 2) over 0.12 s to high swimming velocities typically ranging from 120 cm $s^{-1}$ for the smallest fish to 160 cm $s^{-1}$ for the largest fish (Figure 3). The high speed was then maintained through the burst. The burst was associated with extension of the caudal and decreased tail-beat period. The end of a burst, also marking the start of the coasting phase, was delineated by a rapid decline in swimming speed as the tail was furled and swimming movements usually ceased. The rate of deceleration decreased as the fish approached the current velocity, after which fish velocity relative to the water was zero and the fish was swept backwards at the current speed. The coast ended with the onset of tail beats as fish accelerated in another burst.

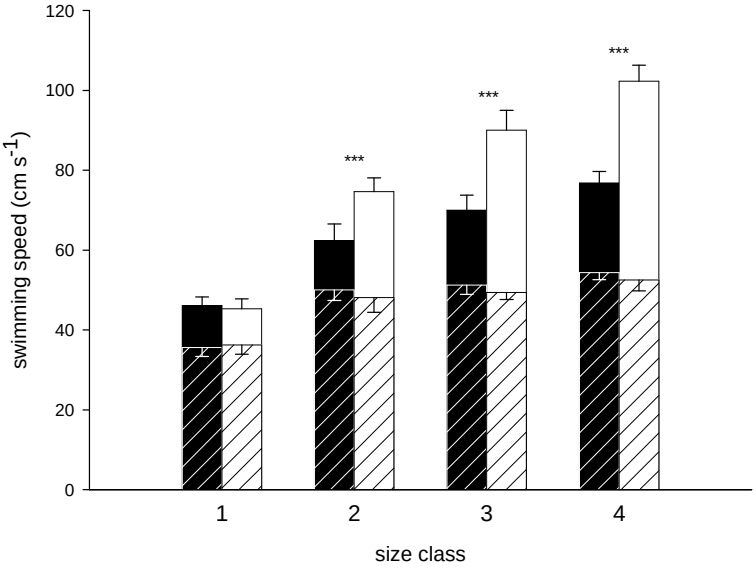


Fig. 1: Critical swimming speed in cm $s^{-1}$ in a short (black) and a long (white) flume, divided in steady gait (striped) and in unsteady burst-and-coast gait, in different size classes of carp. Mean ± SD, N=8.

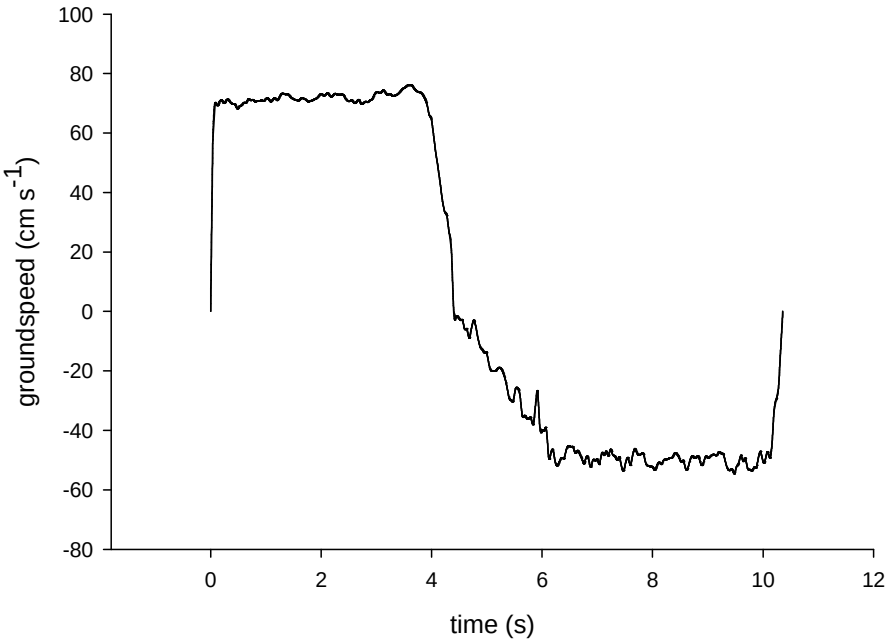


Fig. 2: Observations of fish in flumes are seen from an environmental frame of reference in which bursts are clearly seen as changes in ground speed. Ground speed plotted against time shows a sharp transition to a positive phase of high-speed forward propulsion (burst) and a negative phase of backwards movement (coast). The example is taken from a fish of size class 2, moving in a long flume against a water speed of 50 cm $s^{-1}$.

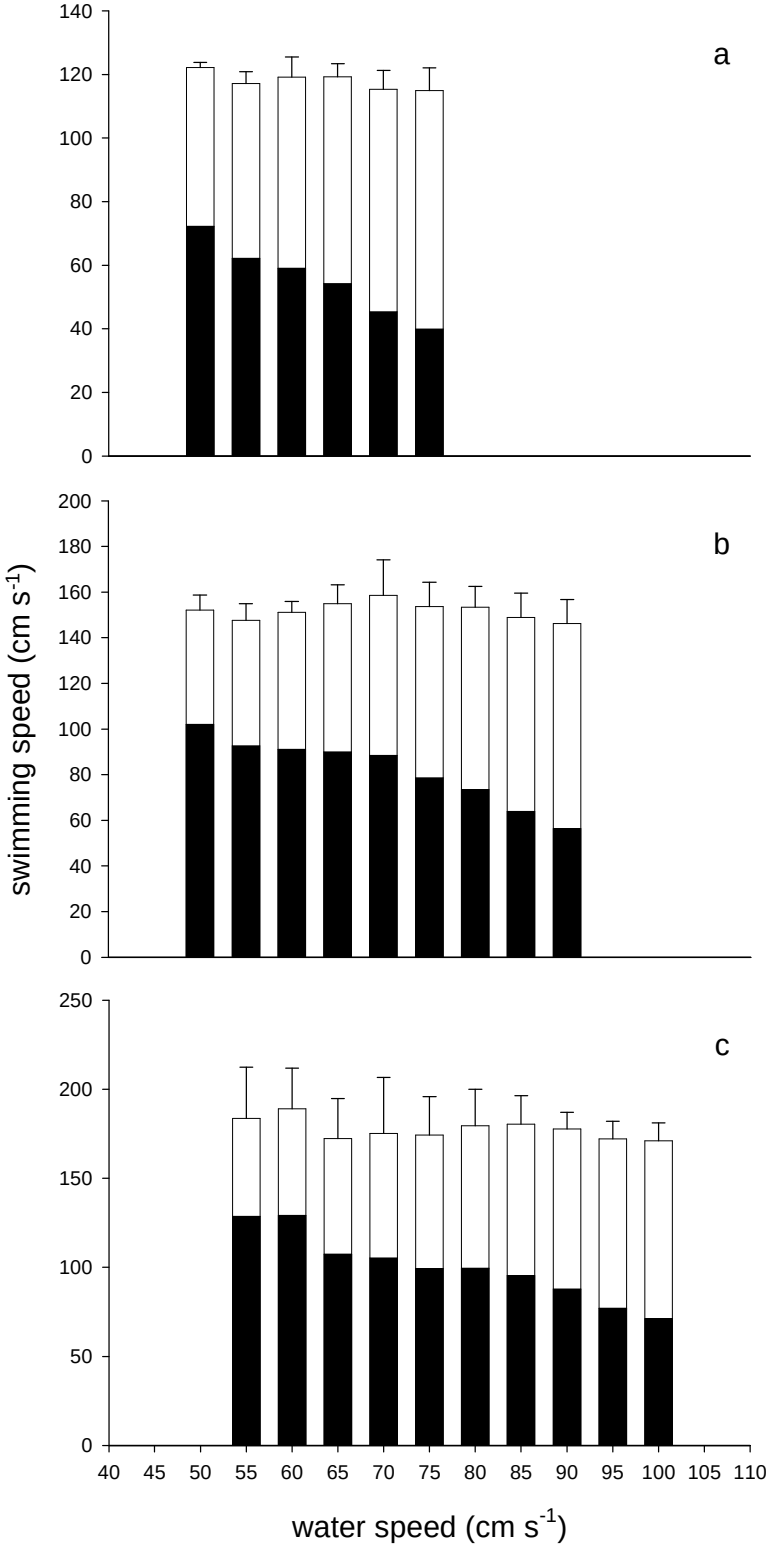


Fig. 3: Average swimming speeds (black) and maximum speeds (white) in cm $s^{-1}$ for size class 2 (10 cm, a), size class 3 (20 cm, b) and size class 4 (25 cm, c) after gait transition. First bar is gait transition speed. Mean ± SD, N=8.

*- Swimming performance -*

Three measures were used to assess effects of chamber length on swimming performance. First, $U_{crit}$ was unaffected by chamber length for the smallest group of fishes (t-test, $p>0.05$), but increased by approximately 20% in groups 2, 3 and 4 from 60 to 80 cm $s^{-1}$ in short chambers to 75 to 110 $cm.s^{-1}$ for the same groups in the long chamber (One-Way Anova, N=8, $p<0.001$). Second, the transition speed from steady swimming to burst-and-coast swimming, expressed as a percentage of $U_{crit}$ was unaffected by chamber length (test and resulting p values) although these transition speeds increased with size (One-Way Anova, $p<0.05$). Third, because $U_{crit}$ increased in the long chamber but transition speed did not, the range of speeds over which burst-and-coast swimming was used also increased in the long chamber. This increase in speed range for burst-and-coast swimming also increased with size in classes 2' 3 and 4 in the long chamber (One-Way Anova, $p<0.001$).

Scaling effects were further explored using linear regression, confirming that body length was positively correlated with $U_{crit}$, transition speed from steady to burst-and-coast gait and the speed range over which burst-and-coast gait was used ($p<0.001$)

The nature of bursts was characterized in terms of their frequency, duration, burst speed and the distance travelled as current speed increased. The frequency of bursts did not change with water speed for all size classes (Figure 4) while the duration of bursts increased. However, the average swimming speed in bursts decreased with increasing current speed (Figure 3;

$p<0.001$) such that the distance travelled decreased. Maximum swimming speeds were greater than $U_{crit}$ and reached approximately 120, 160 and 200cm $s^{-1}$ with size class 2, 3 and 4, respectively. However, although mean speed in a burst decreased with increasing current speed, maxima achieved were independent of current speed. As a result, although fish generally tended to maintain the high

speeds in bursts, their ability to do so declined somewhat with increasing current speed.

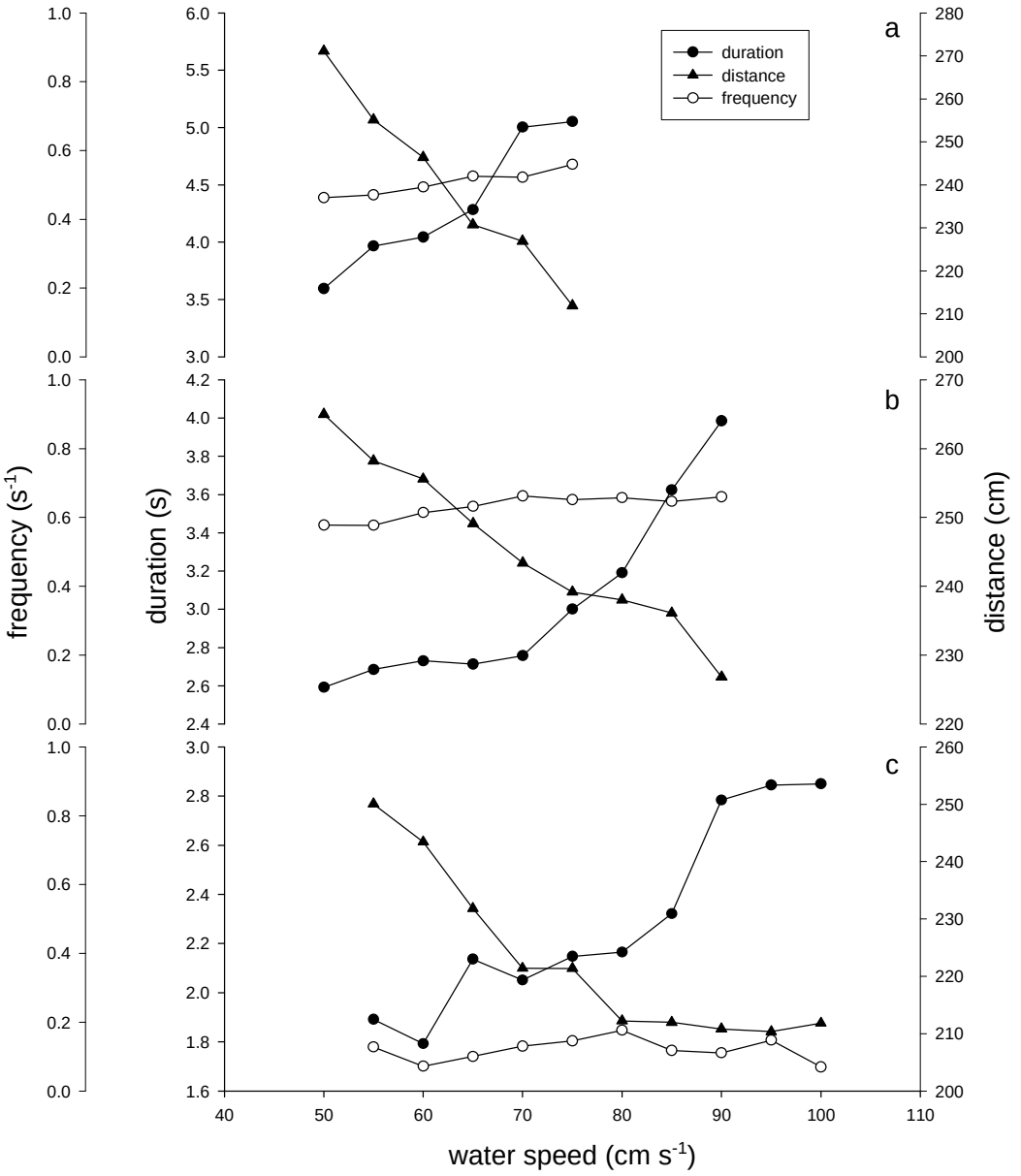


Fig. 4: Analysis of burst and glide movements after gait transition in a three meter flume. Distance (□ ▲), frequency (○) and duration (●) of burst movements are shown across increasing water speeds in the $U_{crit}$ protocol for size class 2 (10 cm, a), size class 3 (20 cm, b) and size class 4 (25 cm, c). Mean ± SD, N=8.

## *Discussion*

The effect of chamber length had various effects on different measures of performance. Thus fish reached higher $U_{crit}$ in longer flumes as also found by Peake & Farrell (2004) and Haro *et al.* (2004), but the transition speed from

steady swimming to burst-and-coast swimming was unaffected by chamber length. Clearly, therefore, chamber length permitted burst-and-coast swimming over a wider range of speeds.

Furthermore, the nature of the increasing velocity test resulted in fish not only swimming at higher speeds but also to swim at these speeds for a longer period of time before being unable to swim off the downstream grid. Therefore, the combination of higher $U_{crit}$ and the longer time over which fish swam at burst-and-coast speeds, fish in the longer chamber would have performed much more work before becoming exhausted. This suggests that the effect of chamber length on performance is not primarily limited by physiological factors determining fatigue, but rather space constraints limiting the ability to execute the behaviour. Certainly space constraints decrease routine swimming rates (Herbert & Steffensen 2005). Furthermore, McFarlane & McDonald (2002) argue that limits on behavioural factors rather than physiological thresholds affect performance for juvenile salmonids.

Nevertheless, physiological factors undoubtedly also contribute to performance. Thus constraints in burst-and-coast swimming were unexpectedly affected by fish size. Short chambers were scaled to fish length while all fish swam in 3-m long chambers. As such, it might be expected that any spatial limitations on burst-and-coast surges would be greater for larger fish in the relatively for larger fish shorter 3-m long chamber. Instead, burst duration of larger fish was longer at a given swimming speed than for smaller fish in spite of the potential constraint on burst distance in the 3-m length chamber. Consequently larger fish covered a larger distance in a burst.

Fish may stop swimming when glycogen reserves reach some threshold. The magnitude of the reserve would be expected to scale with body volume, while the mechanical work performed against resistance would scale with area for viscous-related components. As such, larger fish could have greater stamina,

allowing larger fish to sustain burst-and-coast for longer and hence to higher speeds as observed in these trials.

Finally, larger fish may benefit from the effect of speed increment on $U_{crit.}$ Farlinger & Beamish (1977) showed that $U_{crit}$ is related to the increments of both time and water velocity. Critical swimming speed decreased with increasing time interval, reached a peak and declined thereafter with increasing velocity increment. A fixed speed increment was used, which was, therefore, a smaller size-specific speed increment for larger size classes. Thus the 5 cm $s^{-1}$ speed interval was 1.0 body length $s^{-1}$ for the smallest size class, but 0.25 body length $s^{-1}$ for the largest size class. The smaller size-specific speed increment may have favoured larger fish reaching higher $U_{crit}$.

Overall, irrespective of the cause, general size-dependent effects of chamber length will be factors adding to effects of speed increment and time interval on variation in $U_{crit}$ in the published literature. Similarly, if $U_{crit}$ is considered to be the speed which maximizes oxygen uptake (Webb, 1975; Farrell & Steffensen, 1987; Keen & Farrell, 1994; Gregory & Wood, 1998), longer periods of bust-and-coast swimming probably using anaerobic metabolic pathways might underestimate $\dot{V}O_{2\ max}$ for fish swimming in longer chambers. As it is shown here, the gait transition was not affected by the different swimming chamber lengths, supporting the suggestion by Drucker (1996) that such gait transitions could be better references for comparing metabolic performance among species.

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***Chapter 3***

# *A Comparison of Swimming Capacity and Energy Use in Seven European Freshwater Fish Species*

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*Ecology of Freshwater Fish*; in press

## *Abstract*

Migrating fish species with different swimming capacities and energy use show different capacities for passing obstacles between habitats, such as culverts and fish ladders. Here, we present an integrated study on swimming capacity and energetic use in seven European freshwater fish species with different ranges of migration (brown trout *Salmo trutta* L., European perch *Perca fluviatilis* L., roach *Rutilus rutilus* L., common carp *Cyprinus carpio* L., gudgeon *Gobio gobio* L., bullhead *Cottus gobio* L., stone loach *Barbatula barbatula* L.). Critical ($U_{crit}$), optimal ($U_{opt}$) and maximum ($U_{max}$) swimming speed and oxygen consumption ($MO_2$) were analysed and showed values correlated to migration capacity with highest swimming capacities in trout and roach and lowest in stone loach and bullhead. The resulting data can be used to make estimates of maximum passable water speeds in culverts. In conclusion, long distance migrators show higher swimming capacities and can potentially clear obstacles easier than short distance migrators with lower swimming capacities. Even small obstacles (<25 cm) could be a barrier for genetic exchange between populations in short distance migrators.

## *Introduction*

Swimming performance is one of the crucial factors determining survival within the aquatic environment in fish. Predator-prey interactions, reproduction, migration and dispersal are of great ecological importance and depend on the capacity for locomotion of the individual (Kolok, 1999; Reidy et al., 2000). Therefore, fish species, adapted to different lifestyles and habitats, can be compared in terms of swimming capacity and energy metabolism. With increasing velocity, several swimming speeds are defined: For long distance swimming, fish can swim at optimal swimming speed ($U_{opt}$), i.e. the speed at which the energetic cost of transport is the lowest, powered by aerobic muscles (Brett, 1964; Webb, 1971, 1975; Videler, 1993). Gait transition ($U_{trans}$) marks the transition from aerobically powered steady swimming to aerobically and anaerobically powered unsteady swimming (Beamish, 1978; Videler, 1993). When swimming faster, fish reach critical swimming speed ($U_{crit}$), the speed at which it is generally assumed that maximum oxygen uptake occurs (Webb, 1971; Farrell and Steffensen, 1987; Keen and Farrell, 1994; Gregory and Wood, 1998). Although arguably less informative then other speed definitions in terms of swimming physiology and swimming speeds in the wild, $U_{crit}$ gives a good estimate for swimming capacity in general, as it includes aerobic and anaerobic swimming (Hammer, 1995, Videler 1993). The maximum speed ($U_{max}$), finally, is the white muscle powered speed that can be kept constant only for some milliseconds to seconds and is used for fast and short bursts. This speed is mostly used when escaping from predation or catching prey, but also for short bursts (Howland, 1974; Webb, 1984; Domenici and Blake, 1997; Bergstrom, 2002). With increasing swimming speeds, the sustainability of the swimming modes decreases from $U_{opt}$, which can be kept almost indefinitely, to $U_{max}$ (Videler, 1993; Webb, 1975; Brett, 1964).

When migrating, fish have to pass obstacles on their migration ways. Both long distance and short distance migratory fish species use waterways for migration

(Lucas and Baras, 2001). Physical barriers can obstruct free migration and can cause fragmentation and finally extinction of fish population (Toepfer et al., 1999; Warren et al., 1998; Warren et al., 2000). Often, fish ladders and culverts designed to facilitate the migration are introduced to migratory paths.

Diadromous migration (McDowall, 1997) has ruled until now our perception of mobility in fishes, i.e. non-diadromous species have been deemed to be resident. However, there is growing evidence that most so-called resident freshwater fish species undertake potamodromous migrations, either systematically or in a series of river systems (Lucas and Baras, 2001, Knaepkens *et al.*, 2004, 2005). To our knowledge, swimming capacity and energetics have not been studied in these species, yet. Therefore it is important to gather data on swimming performance and energetics of these species, too, to facilitate management of fish ways.

It has been shown that data obtained in forced swimming experiments cannot be applied without caution in the wild (Castro-Santos, 2004, 2005; Peake and Farrell, 2006). Peake and Farrell (2004) analyzed the swimming behaviour of smallmouth bass (*Micropterus dolomieu*, Lacepede) and showed that at swimming speeds up to $U_{crit}$ bass attained a ground speed of about 0.25 times $U_{crit}$. Thus, if the water speed in a culvert is 0.75 times $U_{crit}$ or less, the fish should be able to pass it over a longer distance. Also, at high swimming speeds ground speed can be close to or equal to the maximum speed less the 0.75 times $U_{crit}$.

The present study compared the swimming capacity and aerobic energy consumption of seven freshwater fish species. The species tested were brown trout *Salmo trutta* L., European perch *Perca fluviatilis* L., roach *Rutilus rutilus* L., common carp *Cyprinus carpio* L., gudgeon *Gobio gobio* L., bullhead *Cottus gobio* L. and stone loach *Barbatula barbatula* L. Also, an estimate of acceptable water velocities for fish passages was given, based on the results of swimming speeds.

## *Material and Methods*

*- Animal holding -*

Fish (bullhead, gudgeon, stone loach, roach, brown trout, carp and perch) of ca. 4 to 11 cm body length (bl) were kept at the University of Antwerp in 200 litre tanks in softened Antwerp City tap water (Ca: 100.8 ± 3.0 mg $l^{-1}$; Mg: 11.0 ± 0.2 mg $l^{-1}$; Na: 36.7 ± 1.2 mg $l^{-1}$; K: 4.6 ± 0.1 mg $l^{-1}$; resulting in a water hardness of 292.4 ± 8.1 mg $l^{-1}$ ($n$ = 30); pH 7.3 ± 0.1; [$O_2$] > 90% saturation; $NH_3$ < 0.1 mg $l^{-1}$ ($n$ = 15)) at a constant temperature of 15 ± 1°C for at least three weeks before experiments started. A flow-through water exchange set up partially renewed the water in the tanks with a turn over rate of 100 litres per day. Additional filtering occurred by means of a circular triple filter consisting of a cotton filter, an active carbon filter and a lava stone filter. Fish were fed with defrosted blood-worms (gudgeon, stone loach, bullhead), pond sticks (Tetra Pond, Germany; common carp, roach) or Nutra Fish Food (Skretting, France; brown trout, perch) three times a week (2 % total body mass) and starved at 24 to 48 h prior to experimentation. Fish of 10 – 30 cm (common carp, roach, perch) were kept at the Flanders Hydraulics Research in Antwerp in 300 litre tanks in rain water at a constant temperature of 15 ± 2°C for at least three weeks before experiments started. The water was permanently filtered by means of a mechanical filter and a closed circuit with a turn over rate of 100 litres per day. Fish were fed with Pond Sticks (common carp, roach) and Nutra Fish Food (perch) three times a week (2 % total body mass) and starved at 24 to 48 h prior to experimentation.

*- Determination of $U_{crit}$ -*

Eight fish from the size groups of approximately 5 to 10 cm (gudgeon, stone loach, common carp, perch, roach and brown trout) were placed in individual separate Blazka-style swimming respirometers with a volume of 3.9 l (Blazka *et al.*, 1960). Bullhead was not included since they successfully anchor themselves

with their pectoral fins at the bottom of the respirometers, and do not swim even at high water speeds. The sizes for the inner tube are 35 x 6 cm and 50 x 11 cm for the outer tube (see Beamish et al. 1989 for comparison). Velocity was set to 5 cm $s^{-1}$. At this speed, the fish orient themselves towards the current and swim gently. For temperature control, respirometers were submerged on a wet table in a room acclimated to the same temperature as the water in the tanks, and a head tank provided a continuous flow of water saturated with oxygen through each respirometer at a rate of 4 l $min^{-1}$ (total volume of the recirculating system approximately 2 x 225 l). These conditions were kept overnight to allow the fish to acclimate to the respirometers. The next day water velocity was then increased in increments of 5 cm $s^{-1}$ at intervals of 20 minutes, until fish fatigued. Fatigue was determined as the situation where the fish could no longer maintain position against the current and were swept downstream. They were held against a mesh screen at the downstream end of the tunnel. Speed was then lowered for a short time to allow fish to restart swimming, and when fish were swept downstream for a third time, they were considered really fatigued and the performance test was terminated.

Eight large fish per species (11 to 20 cm bl; common carp, roach and perch) were tested individually in a Brett-style swimming tunnel (Brett, 1964) with a cross section of 0.4 x 0.4 m, a total of length 20 m and a swimming section of 3 m. The total circulating volume of filtered freshwater was 8000 l. Fish were provided with a 2-hr acclimation period swimming at 5 cm $s^{-1}$. After this period, water velocity was increased automatically in increments of 5 cm $s^{-1}$ at intervals of 20 minutes, until fish fatigued. Fatigue was determined using a photoreceptor-based automated system measuring fish contact with a metal grid delineating the downstream end of the flume. Fatigue was defined as occurring when a fish touched the grid three times in three seconds.

$U_{crit}$ was calculated according to the equation

$U_{crit}=U_i+[U_{ii}(T_i/T_{ii})]$,

with $U_i$ the highest velocity maintained for the whole interval, $U_{ii}$ the velocity increment (here: 5 cm $s^{-1}$), $T_i$ the time elapsed at fatigue velocity, and $T_{ii}$ the interval time (20 min). The absolute values (in cm $s^{-1}$) were converted to relative swimming speeds in body lengths per second (bl $s^{-1}$).

*- $MO_2$ determination -*

Fish (stone loach, gudgeon, bullhead, small carp, small brown trout, small and middle sized roach), were allowed to recover from the $U_{crit}$ determination overnight by swimming constantly at a gentle velocity of 5 cm $s^{-1}$ and respiration measurements were performed the next morning. Respirometry measurements were started by closing the respirometers for a 1-hour period. Oxygen levels never dropped below 70% saturation. During measurements, fish were swimming at different percentages of $U_{crit}$ to determine the $MO_2$ at different swimming speeds using WTW-$O_2$-electrodes (oxi340i, WTW, Germany) connected to the computer program Windmill (Jill Studholme, Windmill Software Ltd, 1996). Oxygen concentrations were then recalculated in oxygen consumption rates in μmol/g/h. After 1 hour of measurement at the lowest speed, respirometers were reconnected to the continuous flow of water saturated with oxygen mentioned above and fish were given one hour to recover. Subsequently the procedure of $MO_2$ measurement was repeated at a higher velocity.

After $U_{crit}$ measurement or $MO_2$ determination was finished, fish were removed and the body length, height and width were measured and fish were weighed. When the calculated cross section of the fish was more that 10% of the swimming tunnel cross section, swimming speed had to be corrected for the solid blocking effect (Bell and Terhune 1970; Herskin and Steffensen 1998), and the following formula was used

$U'=U(1+K)$,

where $U'$ is the corrected swimming velocity $U$, and $K$ is the factor calculated by

$K=\tau\lambda A_0^{1.5}/A_t$,

where $\tau$ is the tunnel shape factor which is 0.8, $\lambda$ is the object shape factor which is 0.5 x (fish body length / body thickness), and $A_0$ and $A_t$ are the cross sectional areas of the fish and of the tunnel, respectively.

*-$U_{opt}$ determination-*

Optimal swimming speed was determined according to Petterson and Hedenström (2000). The exponential function

$$MO_2 = SMR \cdot e^{c \cdot U}$$

is solved for *c*, because when using the exponential metabolic function only *c* will affect $U_{opt}$ and the resulting formula

$$U_{opt} = \frac{1}{c}$$

gives the optimal swimming speed.

For the calculation of the oxygen consumption at $U_{opt}$, the following formula was used

$$MO_{2opt} = SMR \cdot e^{c \cdot U_{opt}}$$

with $MO_{2opt}$ = oxygen uptake at a given velocity (µmol $g^{-1}h^{-1}$) and SMR = standard metabolic rate (µmol $g^{-1}h^{-1}$). *SMR* was determined by extrapolating to zero activity.

To calculate the cost of transport (CoT; in J $N^{-1}$ $m^{-1}$), i.e. the amount of energy a swimming fish spends at a given speed per distance, the formula

$CoT = MO_{2opt}(M.g.U_{opt})^{-1}$

was used with $MO_{2opt}$ = oxygen uptake at $U_{opt}$ (recalculated to mg $O_2$ $g^{-1}s^{-1}$), M = body mass (g), g = acceleration of gravity ($ms^{-2}$) and a oxycaloric value of 14.1 J $(mg\ O_2)^{-1}$ (Videler, 1993).

*- $U_{max}$ determination -*

$U_{max}$ determination was carried out for all fish species, including bullhead, under similar physiochemical conditions as for the $U_{crit}$ determination in small fish. For small fish (5 -10 cm) experiments were carried out in a round white plastic tank with a diameter of 40 cm and a height of 55 cm. For larger fish (10-30 cm), experiments were carried out in a Plexiglas tank of 150 by 150 cm and a height of 50 cm. Water depths was 15 cm for small and 25 cm for large fish, supplied with the same flowing filtered freshwater as in the holding tanks. A reference grid (5 × 5 cm) was drawn on the bottom of the tank for the accurate determination of fish position during escape sequences. Escape responses were induced with a mechanical stimulus, a cubic weight, which was released from a height of 1.5 m above the water surface. Fish were allowed to move freely for at last 15 min before the release of the stimulus. Experiments were carried out at 10, 15 and 20 °C water temperature after a one week acclimation period to the respective temperatures.

A PAL video camera (Sony Corporation DCR-HC39E) was positioned directly above the experimental tank to film the burst swim event. All individuals were filmed three times. Video in PAL consists of 25 frames per second. Each frame can be split into two fields hence the video sequence can be converted into 50 fields per second – 20 ms apart. For this purpose the sequences of escape responses of individual fish were imported into Adobe premiere 6.0 (as AVI files) and deinterlaced. All recordings were analysed, and the three sequences producing the fastest velocities were chosen for further analysis and averaged (Jordan *et al.*, 2005). Analysis was carried out on 20 fields for each individual and commenced on one field prior to the stimulus contacting. The resulting 400 ms were considered sufficient to record maximum velocity (Domenici and Blake, 1997). Each sequence was imported into Vernier Logger Pro 3.3 and the XY coordinates of the centre of mass (CoM) were determined. The CoM was measured by hanging frozen fish from two different points (in

front of the dorsal fin and at the cloaca) and determining the crossing point of the vertical extension of the two lines. Velocity was determined by calculating the movement of CoM from each field over time. Length specific velocity was calculated in bl $s^{-1}$.

*-Estimates for water speeds in culverts-*

Maximum water velocities ($U_{water}$) for culverts were calculated according to Peake and Farrell (2004) using the formula: $U_{water} = U_{max} - 0.75\ U_{crit}$

## *Results*

$U_{crit}$, $U_{opt}$, CoT at $U_{opt}$ and $U_{max}$ (at three temperatures) of *G. gobio*, *B. barbatula*, *R. rutilus*, *C. carpio* and *S. trutta fario* of different sizes are presented in table 1. For *C. gobio*, only $U_{max}$ was determined. Within each species, $U_{crit}$ increased with increasing fish size. The highest values for $U_{crit}$ were found in large perch and large roach, which reached speeds above 1 meter per second. The lowest value was recorded for stone loach. When comparing fish of similar sizes (± 7-10 cm), perch still performed best, followed by trout, carp and roach.

Highest values for $U_{opt}$ were found in gudgeon and lowest values again in stone loach, suggesting that these are not very good swimmers. Values for CoT at $U_{opt}$ were all relatively close together. Trout, together with medium sized roach, showed to be amongst the most efficient swimmers at $U_{opt}$ , with a CoT of 0.26 ± 0.02 $JN^{-1}m^{-1}$. Finally, highest values for $U_{max}$ were found in large roach at 10 °C. At 15°C, gudgeon, large carp and large roach all performed equally well. When comparing equal fish sizes (± 7-10 cm), trout performed better than carp and gudgeon at 10 °C but not at 15°C. Except for the very small roach, all species performed suboptimal at 20°C.

Oxygen uptake at different swimming speeds is given in fig 1. Swimming speeds are presented as percentage critical swimming speed and oxygen uptake ($MO_2$) is given in μmol $g^{-1}h^{-1}$ in order to make values comparable across species. Doing

so, the data represent oxygen uptake at a comparable work load, but not necessarily at a comparable speed. In fish that swim well, such as trout, carp and large roach, the curve is relatively steep, indicating that they can power their increasing swimming speeds by extra oxygen uptake. In other species such as the gudeon, which also reached relatively high $U_{crit}$ values for its small size, the curve remains relatively flat.

The active metabolic rate (AMR) is represented by the oxygen uptake at $U_{crit}$ and values are given in table 2, as are the extrapolations to standard metabolic rate (SMR) and the scope for activity. Standard metabolic rate is lowest for stone loach; in fact values are only a third of the values for roach and trout. Also active metabolic rate is lowest in stone loach, indicating a limited capacity for oxygen uptake in this species. The scope for activity is low in both gudgeon and stone loach. In combination with the high swimming speeds reached by gudgeon, this low scope for activity suggests that perhaps not all of these swimming efforts are powered aerobically. The highest scope for activity was observed in trout and small roach.

In the present study, $MO_2$ and $U$ are assumed to be related in an exponential way as used by Brett before (1964). Other studies have found that $MO_2$ is related to $U$ as other than exponential functions (eg: Videler, 1993). In nature, there is probably not a single mathematical model to describe the relationship of $MO_2$ to $U$ in different species, sizes or ages of swimming fish. However, in order to compare the energetics of the species studied here, a simple exponential equation was appropriate. The $r^2$ remained above 0.96, therefore the model fitted the data well.

*-Estimates for water speeds in culverts-*

Maximum water velocities for culverts were calculated according to Peake and Farrell (2004). Results are only calculated at 15°C since both $U_{crit}$ and $U_{max}$ are required in the calculation. The results are presented in table 1.

Table 1: Body length (bl), critical swimming speed ($U_{crit}$), optimal swimming speed ($U_{opt}$ ), cost of transport (CoT) at $U_{opt}$, maximum swimming speed ($U_{max}$) and recommended maximum water speeds in culverts for bullhead *Cottus gobio*, L., gudgeon *Gobio gobio*, L., stone loach *Barbatula barbatula*, L., roach *Rutilus rutilus*, L., brown trout *Salmo trutta*, L., common carp *Cyprinus carpio*, L., and perch *Perca fluviatilis,* L. Mean ± SD, N = 8

| **Species** | **bl** | **$U_{crit}$** | **$U_{opt}$** | **COT at $U_{opt}$** | **$U_{max}$** | | | **Maximum water speed** |
|---|---|---|---|---|---|---|---|---|
| | **(cm)** | **(cm $s^{-1}$)** | **(cm $s^{-1}$)** | **($JN^{-1}m^{-1}$)** | **(cm $s^{-1}$)** | | | **(cm $s^{-1}$)** |
| | | | | | **10°C** | **15°C** | **20°C** | **15 °C** |
| *C. gobio* | 7.4 ± 0.9 | --- | --- | --- | 112.46 ± 6.72 | 90.43 ± 5.74 | 82.63 ± 3.24 | 61 |
| *G. gobio* | 10.0 ± 0.3 | 54.16 ± 2.02 | 47.09 ± 2.41 | 0.32 ± 0.02 | --- | --- | --- | --- |
| | 12.3 ± 0.3 | 60.17 ± 1.17 | 51.00 ± 2.05 | 0.32 ± 0.03 | 117.61 ± 1.34 | 136.78 ± 1.53 | 116.74 ± 1.87 | 92 |
| *B. barbatula* | 7.2 ± 0.5 | 28.25 ± 0.32 | 18.46 ± 4.47 | 0.28 ± 0.03 | 108.04 ± 1.52 | 83.54 ± 1.46 | 72.73 ± 1.57 | 62 |
| *R. rutilus* | 4.6 ± 0.2 | 45.78 ± 2.10 | 30.93 ± 6.61 | 0.42 ± 0.03 | 55.12 ± 1.37 | 62.37± 0.43 | 64.87 ± 0.26 | 28 |
| | 7.3 ± 0.3 | 59.45 ± 1.27 | 41.49 ± 13.07 | 0.25 ± 0.02 | --- | --- | --- | --- |
| | 15.7 ± 1.5 | 110.75 ± 6.71 | --- | --- | 139.5 ± 1.64 | 133.25 ± 1.53 | 126.00 ± 1.36 | 50 |
| *S. trutta fario* | 7.8 ± 0.2 | 65.43 ± 0.54 | 31.64 ± 0.53 | 0.26 ± 0.02 | 125.86 ± 0.58 | 93.74 ± 0.38 | --- | 45 |
| *C. carpio* | 4.9 ± 0.1 | 43.31 ± 2.15 | 30.59 ± 4.36 | 0.35 ± 0.03 | --- | --- | --- | --- |
| | 10.7 ± 0.2 | 62.30 ± 4.15 | --- | --- | 98.43 ± 0.42 | 103.42 ± 0.34 | 97.34 ± 0.63 | 57 |
| | 22.8 ± 3.9 | 87.09 ± 5.24 | --- | --- | 126.25 ± 1.45 | 134.23 ± 1.52 | 125.42 ± 1.25 | 69 |
| *P. fluviatilis* | 10.1± 0.2 | 80.56 ± 1.50 | --- | --- | --- | --- | --- | --- |
| | 17.8 ± 0.4 | 113.04 ± 1.37 | --- | --- | --- | --- | --- | --- |

Table 2: Body length (bl), standard metabolic rate (SMR), active metabolic rate (AMR) and scope for activity (scope) for gudgeon *Gobio gobio*, L., stone loach *Barbatula barbatula*, L., roach *Rutilus rutilus*, L. and brown trout *Salmo trutta*, L., according to the equation $MO_2 = SMR\ e^{cU}$ with $MO_2$ the specific oxygen consumption over time, SMR the standard metabolic rate, *c* the slope of the resulting curve and *U* the swimming speed. Mean ± SD, N = 8

| | *G. gobio* | | *B. barbatula* | *R. rutilus* | | *C. carpio* | *S.trutta* |
|---|---|---|---|---|---|---|---|
| bl (cm) | 7.4 ± 0.9 | 10.0 ± 0.3 | 7.2 ± 0.5 | 4.6 ± 0.2 | 7.4 ± 0.3 | 4.9 ± 0.1 | 7.8 ± 0.2 |
| SMR (μmol $g^{-1}$ $h^{-1}$) | 7.88 ± 1.53 | 6.1 ± 0.72 | 3.64 ± 0.94 | 9.58 ± 0.21 | 10.84 ± 2.46 | 5.98 ± 0.79 | 10.83±2.37 |
| AMR (μmol $g^{-1}$ $h^{-1}$) | 25.79 ± 3.31 | 22.8 ± 0.66 | 20.1 ± 0.58 | 43.6 ± 0.77 | 34 ± 3.26 | 28.8 ± 1.04 | 50.33±3.21 |
| Scope (μmol $g^{-1}$ $h^{-1}$) | 17.9 ± 2.42 | 16.69 ± 0.69 | 16.45 ± 0.76 | 34.01 ± 0.49 | 23.15 ± 2.86 | 22.81 ± 0.91 | 39.99 ± 1.56 |
| *c* | 0.012 ± 0.003 | 0.013 ± 0.001 | 0.017 ± 0.004 | 0.017 ± 0.003 | 0.012 ± 0.005 | 0.016 ± 0.003 | 0.015 ± 0.007 |

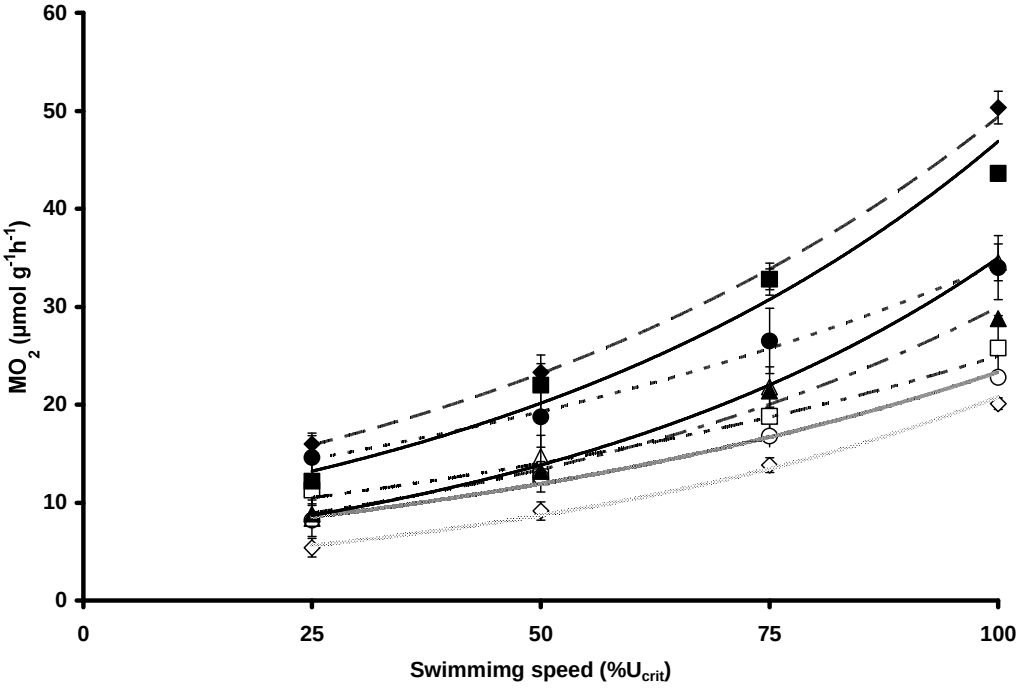


Fig. 1: Oxygen uptake at 25, 50, 75 and 100% critical swimming speed ($U_{crit}$) for gudgeon *Gobio gobio* (7.43 ± 0.93 cm, □; 9.96 ± 0.29 cm, ○), stone loach *Barbatula* barbatula (7.16 ± 0.48 cm, ◊), roach *Rutilus rutilus* (4.60 ± 0.16 cm, ■; 7.36 ± 0.33, ●), common carp *C. carpio* (4.875 ± 0.08 cm, ▲) and brown trout *Salmo trutta fario* (7.84 ± 0.20 cm, ♦). (Mean ± S.D., N=8).

## *Discussion*

The results of the present study present swimming capacities and energy use of different migrating and non-migrating European fish species. As shown, some migratory fish are "better" swimmers than others and display higher swimming speeds or a lower energy consumption while swimming. Trout, roach and carp performed the best in terms of speed and endurance, while gudgeon, loach and bullhead were "weak" swimmers. Unfortunately, we were not able to test large trout since the temperature in the large flume could not be controlled in order for this cold-water species to perform under optimal circumstances. This is also the reason for the low maximum passable water flows calculated for this species (table 1). Jain and Farrell (2003) showed in a repeated swimming performance test that rainbow trout (*Oncorhynchus mykiss*, Walbaum) performed better when acclimated at low temperatures (ca 5°C) than at high temperatures (ca 17°C). However, data on swim performance of salmonids are abundant (Brett, 1964;

Webb, 1975, 1978; Harper and Blake, 1991), and they are excellent swimmers. This was also confirmed in our study, where swimming performances of the small trout were only preceded by small perch. Roach and perch performed excellent as well, especially the larger individuals. This is not surprising since they are typical long distance migratory species (Gerstmeier and Romig, 2000, Vandelanoote et al, 1998, Lucas and Baras, 2001). Unexpectedly, also gudgeon showed high $U_{crit}$ and $U_{max}$ values. Gudgeon is a bottom dwelling species with limited migration behaviour, therefore we did not expect such high values. However, the scope for activity in this species was low. This suggests that a relatively large part of the $U_{crit}$ could have been fuelled by anaerobic metabolism. Therefore long migrations at these high swimming speeds are unlikely for this species. The high $U_{crit}$ and $U_{max}$ indicate that gudgeon has the physiological capacity to pass barriers with fast flowing water, at least when jumping is not required. This has recently been confirmed by observations of gudgeon using a fish pass in the field (Kotusz et al., 2006).

In comparison, stone loach, another bottom dwelling species, performed poorly on critical swimming speed and maximum swimming speed. For technical reasons, $U_{crit}$ could not be measured for *C. gobio*. When set into the swimming tunnel, this species positions its pectoral fins in order to resist the water flow, an adaptation to its natural habitat, where *C. gobio* lives between rocks and stones in fast streaming creeks and small rivers. However, Johnston and co-workers (1995) showed that the main part of Cottidae muscles is glycolytical and this might be an explanation for the typical hopping movement they display. *C. gobio* also lacks a swimming bladder, another adaptation to its environment. Therefore, *C. gobio* does not show any cruise swimming behaviour but always bursts for locomotion. Although *C. gobio* is not generally considered to be a migrating species, there is evidence for migrations of several hundreds of meters by some active individuals (Knaepkens et al, 2004, 2005). Like *C. gobio*, *B. barbatula* also displaces itself by bursts rather than by continuous swimming. New

data indicate that some individuals also perform migrations over several hundreds of meters (Knaepkens, unpublished results). Therefore, species as *C. gobio* and *B. barbatula* are included in our study, and their swimming capacities should be taken into consideration when barriers are remediate by the use of fish passes.

As said by Hammer (2005), Plaut (2001) or Peake and co-workers (1997), critical swimming speed obtained in the laboratory is not a measurement that can be extrapolated directly to populations swimming in the wild but it does give an indication of the swimming capacities of the species tested. In confined spaces such as swimming tunnels, fish alter their behaviour and show very different maximum sustained speeds compared to fish in the wild (Haro et al, 2004; Peake and Farrell, 2006, Tudorache et al, *in press*). However, using the methodology of Peake and Farrell (2004) it is still possible to make fairly reliable predictions about swimming speeds in the wild and passable maximum water speeds, based on $U_{crit}$ and $U_{max}$. The present study aims to give a rough indication for the management of waterways. The $U_{crit}$ data cannot be used to estimate maximum allowable speeds in culverts but based on the obtained data from forced swimming tests, estimation could be made for maximum acceptable velocities in fish passages.

As a fish accelerates in order to pass difficult passages with high water speeds, but also to predate upon prey or escape predators, maximum swimming speeds are used. The results show the capability of some species to accelerate quicker than others. However, species with a higher burst capacity are not necessary more successful in passing difficult passages. For example, *C. gobio* reaches very high burst speeds but is not known to leap out of the water. Without a swimming bladder it is negatively buoyant and remains on the ground. *C. gobio* is not expected to pass obstructions on its migration or dispersion paths. Other species as *S. trutta, R. rutilus* or *C. carpio* are good leapers and can reach long distances and large heights. Those also show good cruising capacities and in

general are more actively moving in the water column. The measurement of $U_{max}$ was done according to traditional methods applied to fast start analysis (Webb, 1975, 1976; Harper and Blake, 1991; Domenici and Blake, 1997) and was not taken from a natural situation in the field, implying linear bursts. However, the data obtained from such measurements were used before to make estimates of bursting and leaping capacities in freshwater fish (e.g.: Wolter and Arlinghaus, 2003; Videler, 1993). The duration of 400 ms might not be sufficient to reach maximum speeds when comparing to situations in the field but the maximum velocities obtained from such analysis can give a good estimate of maximum possible speeds when bursts are implied in clearing obstacles on migration paths and in general for an estimation of anaerobic swimming capacity.

Optimal swimming speed is the speed at which the cost of transport is lowest. It is a theoretical value, and there is no evidence for the actual use of this swimming speed in nature. Moreover, as fish pass difficult areas on migration routes, speeds are often altered and energy saving strategies like burst-and-coast swimming is adopted, as this swimming behaviour has been proven to reduce aerobic energy demands for up to 60% (Weihs, 1974). Hinch and Bratty (2000) showed that migrating Sockeye Salmon (*Oncorhynchus nerca*, Walbaum) never swam at expected, i.e. energy saving speeds. Especially while passing difficult passages on their migration route, burst-and-coast swimming was adopted. Nevertheless, the use of $U_{opt}$ allows a comparison of the speed and cost of steady swimming between and within species. The data show that smaller fish swimming at $U_{opt}$ swim slower than bigger fish of the same species, while they use a higher percentage of their scope for activity. This means that they have a higher CoT at $U_{opt}$ and use more energy per meter swum. Thus, being a larger fish is energetically more advantageous.

When comparing swimming velocities of different fish species and sizes speed per unit body length is often applied (Videler and Wardle, 1991; Drucker, 1996; van Damme and van Doren, 1999; Drucker and Lauder, 2000; Plaut, 2001).

However, Drucker (1996) found 'per unit body length speed' caused errors in the kinematic and physiological comparison of exercise between different fishes. Furthermore, studies by Kolok, (1999) and Reidy *et al.* (2000) demonstrated significant variability of locomotor performance between individual fish. However, to evaluate swimming performance of fish with regard to the physical clearance of obstructions on migration paths, absolute values of swimming speed seem appropriate, because water velocities in culverts and fish ladders are absolute values. Thus, each individual fish has to withstand a certain threshold value, independent of the kinematic and physiological comparability of its exercise performance.

As swimming performance and energetics are different in different fish species, all fish species should be considered when evaluating possible effects of barriers. Man made obstacles on the pathways of migrating fish species can be one of the factors reducing migration behaviour and thus leading to fragmentation and finally extinction of fish species. This study shows that typically migratory species such as trout, perch and roach perform best in the swim performance tests. Additionally, also gudgeon perform surprisingly well. It is shown that besides bullhead, which is already threatened in Flanders, stone loach is a species of concern when barriers, even small ones, are present.

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***Chapter 4***

# *Swimming Capacity and Energetics of Migrating and Non-Migrating Morphs of Three-Spined Stickleback (Gasterosteus Aculeatus L.) and their Ecological Implications*

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## *Abstract*

The two morphotypes (leiurus and trachurus) of the three-spined stickleback (*Gasterosteus aculeatus* L.), caught at the same location and time in the River Scheldt (Belgium), were investigated for physiological differences in swimming capacity and energetics associated with migration. Critical and optimal swimming speeds, maximum speed and gait transition speed were significantly higher for the trachurus type. Standard metabolic rate and active metabolic rate were also higher for trachurus, as was scope for activity. Energy stores (protein, lipid, and glycogen in liver and white muscle) were mostly similar in the two types, but lipids in trachurus liver tissue were significantly higher.

## *Introduction*

The three-spined stickleback (*Gasterosteus aculeatus* L.) is one of the most abundant fish species in the coastal waters of Europe because it is a pioneer species (Gerstmeier & Romig, 2000). Two morphotypes, leiurus and trachurus, exist. The leiurus type, morphologically distinguished by a row of small scales providing armour over the anterior half of the body, does not migrate in Northern Europe but spends the migration period (April to July), typical of the migratory form, in shallow fresh or brackish waters where spawning occurs. The trachurus type is anadromous in Northern Europe and is characterised by strong armour over the whole body and a pair of horizontal keels at the base of the tail fin. Trachurus types migrate from marine or estuarine environments to spawn in streams, and may travel long distances to reach fresh water (Vandelanoote *et al.*, 1998; Gerstmeier & Romig, 2000; Lucas & Baras, 2001).

The accumulation of energy stores in trachurus varies with seasonality to meet the demands of spawning migration in spring. Migration has substantial effects on energy metabolism (Slotte, 1999; Lee *et al.*, 2003; Mommsen, 2004; Aas-Hansen *et al.,* 2005). Since European sticklebacks comprise both a migratory and resident morph within the same population, the species is an ideal model to answer important questions on physiological adaptations related to migration.

The aim of the study was to compare swimming performance, energy use, and energy stores in the two morphotypes. Variables investigated were; (*i*) critical swimming speed ($U_{crit}$), defined as the nominal speed at which maximum oxygen uptake occurs (Webb 1971; Farrell & Steffensen 1987), (*ii*) gate transition speed ($U_{trans}$), i.e. the swimming speed at which the fish changes from steady to burst and coast swimming, (*iii*) maximum swimming speed ($U_{max}$) during escape, (Beamish 1978; Videler 1993; Domenici & Blake, 1997) (*iv*), optimal swimming speed ($U_{opt}$), defined as the speed at which the lowest relative oxygen uptake occurs, (Tucker, 1970; Videler, 1993) and (*v*) scope for activity, calculated as the difference between the standard metabolic rate (SMR) and the active metabolic

rate (AMR), giving an indication of the exercise range at which a fish can function aerobically. The hypothesis was that the trachurus type would show higher swimming performance, combined with a larger scope for activity, and elevated tissue energy stores during the migration period, when compared to the leiurus type.

## *Material and Methods*

### *- Animal Holding -*

Fish were caught using fish traps in the River Sheldt near Ghent (Belgium) in the beginning of March 2005 and transported to the University of Antwerp. Fish were placed in 200 l holding tanks containing softened, mechanically and biologically filtered Antwerp City tapwater. They were held at 15° C in ambient photoperiod for March (12:12 LD) for at least 14 days before the experiment began. The water was partially renewed at 100 l per day. Fish were fed with defrosted blood-worms three times a week (20 % total body mass) and starved at 24 to 48 h prior to experimentation.

### *- $U_{crit}$ and $U_{trans}$ Determination -*

For determination of $U_{crit}$, eight fish of each morph were placed individually in separate Blazka-style swimming respirometers, volume 4.1 l. The sizes of the inner and outer tubes were 35 x 6 cm and 50 x 11 cm, respectively (see Beamish *et al.*, 1989). Velocity was set to 5 cm $s^{-1}$. At this speed, the fish orientates itself against the current and swims steadily. Each respirometer was supplied with water (15° C) at a rate of 4 l $min^{-1}$ (total volume of the recirculating system was approximately 450 l). These conditions were maintained overnight to allow the fish to acclimate. Beginning about 20 h later, velocity was increased in increments of 5 cm $s^{-1}$ at 20 min intervals, until the fish fatigued. Fatigue was determined as the point where the fish could no longer maintain its position against the current and was swept against a mesh screen downstream of the

tunnel. The speed was then briefly reduced to allow the fish to resume swimming. When fish were swept downstream for a third time, they were considered fully fatigued and the test was terminated. $U_{crit}$ was calculated according to the equation

$$U_{crit}=U_i+[U_{ii}(T_i/T_{ii})],$$

with $U_i$ being the highest velocity maintained for the whole interval, $U_{ii}$ the velocity increment (here: 5 cm $s^{-1}$), $T_i$ the time elapsed at fatigue velocity, and $T_{ii}$ the interval time (here: 20 min; Brett, 1964). The absolute values (cm $s^{-1}$) were converted to relative swimming speeds in fork lengths per second ($L_F$ $s^{-1}$). The swimming fish was observed, and the speed at which the first three transitions from cruise swim to burst and coast swim occurred was noted and defined as $U_{trans}$. The burst was associated with extension of the caudal fin and decreased tail-beat period.

*- Determination of Oxygen Consumption ($MO_2$) -*

The fish were allowed to recover overnight from the $U_{crit}$ experiment in water circulating at a velocity of 5 cm $s^{-1}$. Respiration measurements were performed the following morning. Oxygen consumption was determined at 25, 50, 75 and 100% $U_{crit}$. Oxygen was measured with WTW-$O_2$-electrodes (oxi340i, WTW, Germany) connected directly to a computer for recording (Windmill program, Jill Studholme, Windmill Software Ltd, 1996). After 1 h at the lowest speed, respirometers were reconnected to the continuous flow of water which was saturated with oxygen, and the fish were given 1 h to recover. Subsequently, the measurement of $MO_2$ was repeated at a higher velocity. The oxygen concentrations were calculated as oxygen consumption rates in µmol $g^{-1}$ $h^{-1}$. At the completion of $MO_2$ measurements, the fish were removed, weighed, and volume and fork length ($L_F$) determined. The condition factor (K) according to Fulton (1902) was calculated, using the formula

$$K_F = 100M / L_F^3$$

with $M$ being body mass in g and $L_F$ fork length in cm.

*- $U_{opt}$ Determination -*

The optimal swimming speed, *i.e.* the speed at which the lowest oxygen uptake per unit distance swum occurred, was determined according to Petterson & Hedenström (2000). The exponential function

$$MO_2 = SMR \cdot e^{c \cdot U}$$

was solved for $c$, because when using the exponential metabolic function only $c$ will affect $U_{opt}$ and the resulting formula

$$U_{opt} = \frac{1}{c}$$

with $U_{opt}$ = the optimal swimming speed (cm $s^{-1}$) and $c$ = constant (s $cm^{-1}$), gave the optimal swimming speed.

For calculating oxygen consumption at $U_{opt}$ ($MO_{2opt}$), the following formula was used

$$MO_{2opt} = SMR \cdot e^{c \cdot U_{opt}}$$

with $MO_{2opt}$ = oxygen uptake at a velocity $U_{opt}$ (µmol $g^{-1}h^{-1}$) and $SMR$ = standard metabolic rate (µmol $g^{-1}h^{-1}$).

To calculate the cost of transport ($CoT$; in J $N^{-1}$ $m^{-1}$), i.e. the amount of energy a swimming fish spends at a given [speed per distance], the formula

$CoT = MO_{2opt}(M.g.U_{opt})^{-1}$

was used with $MO_{2opt}$ = oxygen uptake at $U_{opt}$ (recalculated to mg $O_2$ $g^{-1}s^{-1}$), $M$ = body mass (g), $g$ = acceleration of gravity ($ms^{-2}$) and a oxycaloric value of 14.1 J mg $O_2^{-1}$ (Videler, 1993).

*- $U_{max}$ Determination -*

Evaluation of $U_{max}$ was carried out in a circular white plastic tank, diameter 40 cm and height 55 cm, containing filtered flowing fresh water (as in holding tanks) to a depth of 15 cm. A 5 cm × 5 cm reference grid was drawn on the

bottom of the tank for accurate determination of fish position during escape sequences. Escape responses were elicited by dropping a cubic weight into the tank from a height of 1.5 m above the water surface. The fish were allowed to move freely for at last 15 min before the release of the stimulus. Each fish was subjected to the experiment three times at 20 min intervals.

A PAL video camera (Sony Corporation DCR-HC39E) was positioned directly above the tank. The three trials were filmed at 25 frames $sec^{-1}$. Each frame was split into two fields for 50 fields $sec^{-1}$. The sequences were imported into Adobe premiere 6.0 as AVI files and de-interlaced. All recordings were analysed, but only the sequence producing the fastest velocity was chosen for further analysis (Jordan *et al.*, 2005). The analysis was carried out on 20 fields for each individual and began one field prior to the weight contacting the water. Each sequence was imported into Vernier Logger Pro 3.3, and the xy coordinates of the centre of mass (CoM) were determined. Velocity was determined by calculating the position of CoM from in each field over time. Length specific velocity was calculated in $L_F\ s^{-1}$.

*- Biochemical Analysis -*

The fish were killed with a lethal dose of MS 222 (1g $l^{-1}$, pH 7.5) and liver and white muscle tissue were removed, weighed, and stored immediately at -20° C. Total lipids were extracted from both liver and muscle following Blingh & Dyer (1959) and measured using a tripalmitin standard curve. Total protein was determined according to Bradford (1976), using a standard curve of bovine serum albumin (US Biochemical, Cleveland, OH, USA). Glycogen was determined with Anthrone reagent (Roe & Bailey, 1966) using a glycogen standard curve (Merck, Leuven, Belgium). The energy reserve fractions for the individual fish were transformed into energetic equivalents using the enthalpy of combustion (Gnaiger, 1983): 17 500 mJ $mg^{-1}$ glycogen, 24 000 mJ $mg^{-1}$ protein and 39 500 mJ $mg^{-1}$ lipid.

*- Statistical Analysis -*

Statistical analysis was performed by one way ANOVA (GraphPad InStat version 3.05). When $P < 0.05$, Tukey was used as a post hoc test.

## *Results*

*- Fork Length, Mass, and Condition Factor -*

The difference in mean $L_F$ between the two populations was not significant (5.44 ± 0.14 cm for trachurus and 5.28 ± 0.12 cm for leiurus), while mass (*M*) was significantly different: 3.33 ± 0.15 g for trachurus and 2.59 ± 0.21 g for leiurus ($P < 0.05$). The condition factors also were significantly different: 2.18 ± 0.15 in the trachurus and 1.72 ± 0.14 in the leiurus population ($P < 0.05$).

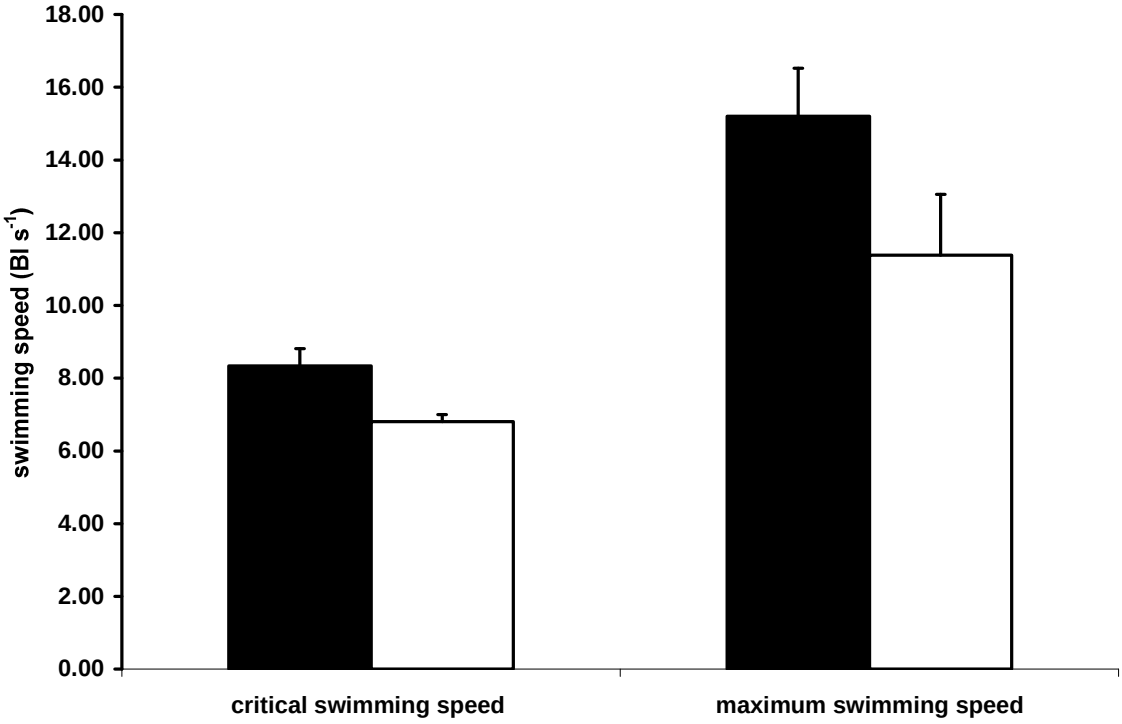


Fig. 1: Critical and maximum swimming speeds of trachurus (black) and leiurus (white) *Gasterosteus aculeatus*. (Mean ± S.D., n=8).

*- Swimming Performance and Respirometry -*

Critical swimming speeds ($U_{crit}$) were 22% higher in trachurus than in leiurus ($P < 0.001$ Fig. 1). Maximum swimming speed ($U_{max}$) also reached significantly higher values in trachurus (25%, $P < 0.001$, Fig. 1). Oxygen consumption differed significantly at 100% $U_{crit}$. The $MO_2$ value at 100% $U_{crit}$ (active metabolic rate, AMR) in trachurus was 40% higher than in leiurus ($P < 0.001$, Fig. 2).

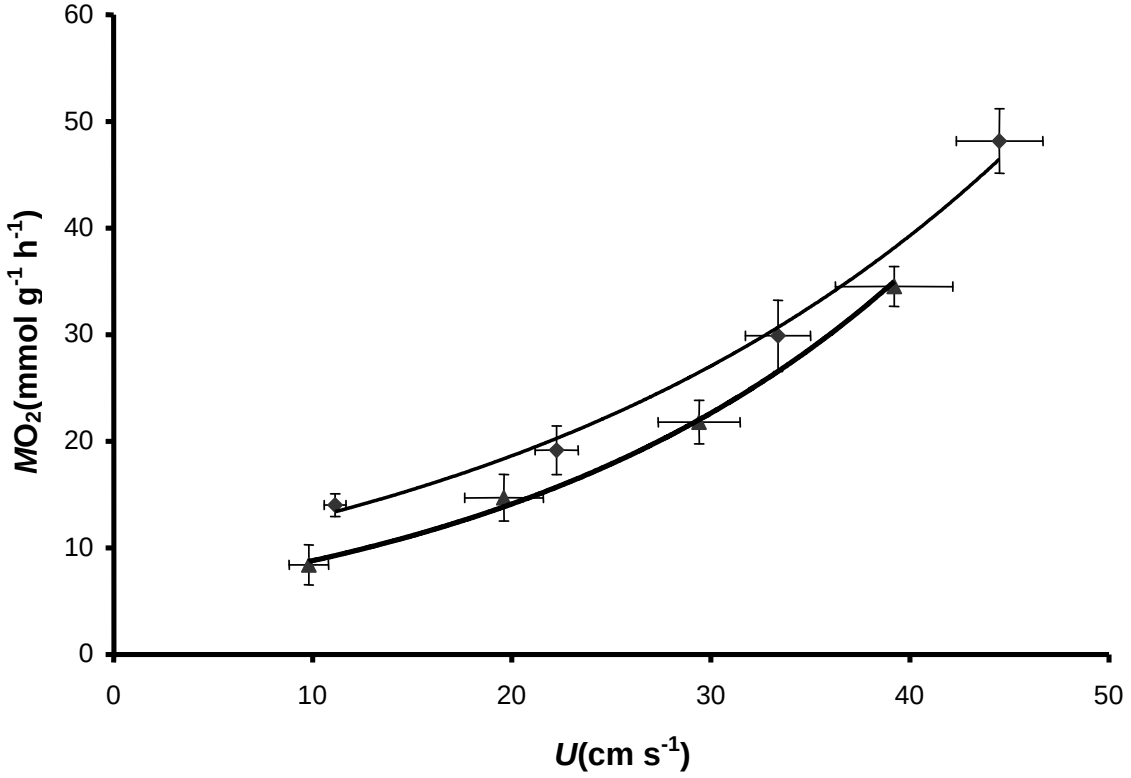


Fig. 2: Oxygen uptake at 25, 50, 75 and 100% critical swimming speed ($U_{crit}$) for trachurus (♦) and leiurus (▲) *Gasterosteus aculeatus*. Optimal swimming speed ($U_{opt}$) and oxygen uptake at optimal swimming speeds ($MO_{2opt}$) are indicated for trachurus (continuous line) and for leiurus (dotted line) types. Tangents are visualising the geometrical approach to $U_{opt}$ calculation. (Mean ± S.D., n=8).

Extrapolation to zero revealed a standard metabolic rate (SMR) in trachurus of 8.84 ± 0.94 μmol $g^{-1}$ $h^{-1}$ and in leiurus, 5.62 ± 1.50 μmol $g^{-1}$ $h^{-1}$. The scope for activity (i.e. the difference between AMR and SMR) was 39.32 ± 3.72 μmol $g^{-1}$ $h^{-1}$ for trachurus and 28.91 ± 2.37 μmol $g^{-1}$ $h^{-1}$ for leiurus. All differences were

significant ($P < 0.05$). Optimal swimming speed was calculated from respirometry curves and reached 26.71 ± 3.37 cm $s^{-1}$ (4.96 ± 0.38 $L_F$ $s^{-1}$) with a correlating respiratory value of 24.04 ± 2.37 μmol $g^{-1}$ $h^{-1}$ in trachurus, and 22.01 ± 4.91 cm $s^{-1}$ (4.16 ± 1.04 $L_F$ $s^{-1}$) with a correlating respiratory value of 15.27 ± 4.07 μmol $g^{-1}$ $h^{-1}$ in leiurus. The differences were significant ($P < 0.05$). There was no significant difference in cost of transport $CoT_{opt}$ between the morphs (0.41 ± 0.09 J $N^{-1}m^{-1}$ in trachurus and 0.48 ± 0.13 J $N^{-1}m^{-1}$ in leiurus).

*- Energy Stores -*

Data on energy stores are shown in Table 1. Significant differences were found between the two populations only for liver lipid content ($P < 0.05$).

Table I. Lipid, glycogen and protein content of liver and white muscle tissue in J $g^{-1}$ for trachurus and leiurus *Gasterosteus aculeatus* individuals with significantly higher liver lipid levels in trachurus (*) than in leiurus. (Mean ± S.D., n=8)

| | Trachurus type | | | Leiurus type | | |
|---|---|---|---|---|---|---|
| | Lipid (J $g^{-1}$) | Glycogen (J $g^{-1}$) | Protein (J $g^{-1}$) | Lipid (J $g^{-1}$) | Glycogen (J $g^{-1}$) | Protein (J $g^{-1}$) |
| Liver | 5580 ± 407* | 346 ± 39 | 2759 ± 266 | 5096 ± 405* | 304 ± 20 | 2497 ± 117 |
| Muscle | 2359 ± 974 | 308 ± 29 | 1452 ± 43 | 1719 ± 506 | 291 ± 27 | 1424 ± 30 |

## ***Discussion***

The two morphs showed significant differences in all swimming performance characteristics measured. Higher values of $U_{crit}$, $U_{opt}$ and $U_{max}$ were found in trachurus than in leiurus. Differences in $U_{max}$ performance between types of sticklebacks have previously been reported in North American populations (Law & Blake, 1996) which also show similar specialisation, inhabiting different niches and foraging on different resources (Hart & Gill, 1994). Specialisation in the North American sticklebacks shows a continuum from benthic to limnetic (Rogers, 1968; Lavine & McPhail, 1986). Their swimming performance also differs from their European anadromous relatives (Law & Blake, 1996). As Law

& Blake (1996) pointed out, the benthic, less-armoured North American sticklebacks reached higher burst speeds when startled. In contrast to Bergstrom (2002), the stronger armoured trachurus individuals showed higher burst speeds compared to the soft-bodied leiurus types.

European trachurus sticklebacks require the ability to escape predators but also rely on body armour to withstand predator attacks. They may not show the same flexibility as the non-migrating leiurus individuals for fast starts involved in escaping predators or predation. Despite the rigid body form of trachurus, this morphotype reached higher values of $U_{crit}$ and $U_{max}$ compared to leiurus. These results do not support a theoretical assumption that trachurus are adapted for higher sustained swimming, and show a poorer capacity for predator avoidance (*i.e.* fast starts), as stated by Bergstrom (2002).

Migration to spawning grounds requires upstream swimming. It might be assumed that fish migrate at the speed with the lowest cost, at $U_{opt}$. In the present study, $U_{opt}$ differed significantly between the two morphs, with trachurus showing higher values than leiurus. This would indicate that trachurus can swim at faster migration speeds at a relatively lower cost (*i.e.* energy consumed per distance swum). Swimming at $U_{opt}$, trachurus types reached higher speeds than leiurus but consumed a greater amount of oxygen in absolute terms, which would seem unfavourable from a theoretical perspective. Yet the percentage increase in $\dot{M}O_{2opt}$ compared to the total scope of activity between the two morphs was identical, indicating that the same percent of their aerobic energy budget was directed towards swimming at $U_{opt}$. Also, $CoT_{opt}$ was not significantly different, indicating that when swimming at the same speed, both morphs expended the same amount of energy, and that the elevated $\dot{M}O_{2opt}$ value was only due to the increased SMR in trachurus. A possible reason for the higher energy demand at rest in migratory trachurus might be an increase in blood circulation carrying more nutrients and oxygen to the muscles, possibly due to faster heart rates. However, it has not been proven that fish swim at $U_{opt}$ while

migrating (Standen *et al.*, 2002). $U_{opt}$ is a theoretical approach giving an indication of the oxygen uptake curve across swimming speeds and is therefore of interest in fish bioenergetics.

Extrapolation to zero swimming speed showed higher values of SMR in trachurus compared to leiurus. The higher SMR in the migrating group agreed with a previous study and reflected the values found for migrating sticklebacks in summer, while the data for leiurus reflected the values found for sticklebacks in winter, during the non-migration period (Meakins, 1975). Meakins (1975) also found a proportionately lower scope for activity in leiurus than in trachurus. By adjusting the SMR, fish could compensate for the energy demands of migration (Petterson & Hedenström, 2000), by allowing them, as is the case with trachurus, to reach a higher AMR.

The method of determining the SMR by extrapolating to zero velocity may bias estimates, and SMR could be overestimated if the swimming speed and $MO_2$ functions were elevated, or the regression slope was reduced due to inefficient swimming at low speeds (Brett, 1964). However, Schurmann & Steffensen (1997) observed no difference between extrapolated values of SMR and SMR measured in resting fish at three different temperatures. Moreover, the two morphs of the same species were treated identically. Any error arising using this method should be the same for both morphs and thus ignorable.

Even though the fish were held under identical conditions and were fed sufficiently, trachurus showed higher liver lipid content compared to leiurus. There were no differences in protein content. With protein it is mainly the turnover rate and not the absolute concentration that changes (Jürss *et al.,* 1997; Guderley *et al.* 2001; Huntingford *et al.,* 2001; Guderley *et al.*, 1994). However, it cannot be ruled out that the time of capture may play a role in the body composition differences found in this study. It therefore remains an open question whether trachurus has generally higher liver lipid reserves or just a seasonally higher physiological capacity to store lipids compared to leiurus.

Smolders *et al.* (2003) showed a positive correlation between energy reserves and swimming performance in zebrafish (*Danio rerio* Harrison*)*. The present results indicate that higher levels of lipids may be an adaptive trait of trachurus related to migration.

Comparative studies of freshwater fish migration must consider seasonal and temporal factors (see Lucas & Baras, 2001) which can make results difficult to interpret**.** The three-spined stickleback is a good model for studying migrating and non-migrating types from the same habitat at the same time.

***Acknowledgements***

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***Chapter 5***

# *The Amount of Trunk Undulation Increases Turning Radii with Increasing Speed*

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## *Abstract*

Manoeuvrability of a swimming fish, defined as minimum turning radius per swimming speed, depends on body undulation and flexibility. Anguilliform (eel, *Anguilla anguilla*), subcarangiform (sea bream, *Abramis brama*) and labriform swimming fish (striped surfperch, *Embyotoca lateralis*) show decreasing body flexibility when swimming, using less surface of their body for propulsion. When plotting minimum turning radius against swimming speed, the slope of the resulting line decreases with increasing body flexibility, showing the lowest values with eel and the highest values with striped surfperch. This indicates that turns are becoming less tight with higher speeds in rigid body labriform swimming compared to flexible body anguilliform swimming fish and therefore, manoeuvrability at high speeds is reduced.

## *Introduction*

Fish use their body and fins for swimming in many different ways and combinations. It can be generalised that fishes either use median fins and paired fins (MPF) or the body and caudal fins (BCF) for swimming (Videler 1993). MPF propulsors especially in labriform swimmers (Breder 1926) are used at low speeds, where efficiency can be higher compared with BCF propulsors, especially in a dense habitat (Webb 1993). BCF propulsors may be used at high speeds and accelerations in an open habitat as they provide a greater power output (Webb 1984). For BCF swimmers the performance depends on the extent the body undulates during swimming. Fish species using about the half of their trunk region to undulate are called subcarangiform swimmers. Angulliform swimmers use almost the whole of their trunk region (Breder 1926).

While the importance of steady locomotion for survival is widely appreciated, unsteady locomotion is also important (Biewener and Gillis, 1999; Blob et al., 2006). For example, animals living in a complex habitat or engaged in predator-prey interactions need to change direction frequently to avoid obstacles or evade predators or capture food. Therefore, turning performance is an important aspect of locomotion for many species (Howland, 1974; Gerstner, 1999; Domenici, 2001; Hedenström and Rosén, 2001).

Tight turns are characteristic features of fish manoeuvring skills, but few studies have measured how tightly fish can actually turn (Domenici & Blake, 1991, Webb, 1983, Videler, 1993). Swimming fish are limited in the flexibility of the curvature of their swimming paths, expressed as turning radius ($R_T$, Domenici & Blake, 1997). The minimum $R_T$ of a fish depends on body flexibility. A small $R_T$ can be beneficial for both predator and prey, but it is also useful in complex environments such as coral reefs or densely vegetated freshwater systems.

The objectives of this study aim to correlate $R_T$ with swimming speed in fish with increasing flexibility of the body and increasing use of the trunk region for propulsion.

## Material and methods

### Fish

Striped surfperch: Fish of 18.6 ± 0.4 cm (large) and 14.3 ± 0.5 cm (small) were collected by beach seining at Jackson Beach off the San Juan Island. The study was carried out at Friday Harbor, University of Washington, USA. Fish were maintained unfed for at least 72 h prior to experimentation in holding tanks, receiving a constant flow of seawater at a temperature range of 13.0 ± 0.5 ºC. Light/dark regime was natural. All fish were kept according to the U.S. laws on animal welfare and were released unharmed at the end of the study.

Eel: Fish of 56 ± 14 cm $L_B$ (n = 10) were obtained from a commercial eel farm (Royaal BV, Helmond, The Netherlands). Fish were kept at the University of Leyden, The Netherlands, in a 180 l tank connected to a 2200 l recirculation system in artificial seawater (35ppt, 18˚C) under a 12/12 h light/dark regime. PVC pipes were added to serve as shelter.

Seabream: Fish of 19.5 ± 2.5 cm were supplied by BioMar A/S located in Hirtshals (Denmark) and transported in well-aerated tanks to the Marine Biological Laboratory in Helsingør (Denmark). They were kept at 20ºC in 700 l holding tanks continuously supplied with well-aerated, full-strength seawater. The light condition was a 16/8 h light/dark regime.

### Experiments

Seabream, Striped surfperch: The 2D position of a fish was registered at a rate of 25 frames $s^{-1}$ by a CCD-camera (TVCCD 460; Monacor, Denmark) mounted above an perspex circular arena (diameter 41.0 cm, height 11 cm) as described by Steinhausen (2005). Images were digitized with a video capture card (Pinnacle PCTV Rave) with a resolution of 640x480 pixels. Labtech Notebook Pro was used for data acquisition via a Measurement Computing PCMCIA-DAS16D/D interface board. Experiments were carried out at dim light conditions, and an infra red light source (Monacor IR-10) illuminated the fish from below. The

tracking of the fish was determined by the principle of contrast, *i.e.* the fish was tracked against a contrasting background and LoliTrack software (Loligo Systems, Hobro, Denmark) assigned a *xy* coordinate pair to the geometrical centre of mass of the contrasted fish at a frequency of 5 Hz. Alternatively, a PAL video camera (Sony Corporation DCR-HC39E) was positioned directly above the experimental tank to film the swimming fish at a frequency of 25 fps. Each sequence was imported into Vernier Logger Pro 3.3 and the XY coordinates of the centre of mass (CoM) were determined manually. In order to evaluate possible effects of different set ups used, striped surfperch of 18 cm $L_B$ was filmed in a small (diameter 41.0 cm, height 11 cm) and a large circular arena (diameter 74 cm, height 55 cm). Also, to avoid an effect of different analysing methods, the same sequence taken of a surfperch with XX $L_B$ by automatic videotracking was additionally imported into Vernier logger pro 3.3 and the XY coordinates of the CoM were determined manually. There were no significant differences for any values analysed.

Eel: Fish were introduced into an experimental tank of 185 cm x 100 cm x 50 cm. A PAL video camera (Sony Corporation DCR-HC39E) was positioned directly above the experimental tank at a height of 1.53 m to film the swimming fish at a frequency of 25 fps. Each sequence was imported into Vernier Logger Pro 3.3 and the XY coordinates of the CoM were determined manually.

The velocity ($U$) was determined by calculating the movement of CoM from each field over time. The turning radius ($R_T$) was determined according to Domenici and Blake (1997) as the radius of the circle that can be calculated from three consecutive positions of the centre of mass.

*Data analysis and Statistics:* XY coordinates were used to calculate velocity ($U$) as distance per time interval and Turning Radius ($R_T$) according to Domenici & Blake (1997). Resulting data were filtered to avoid random noise and lowest $R_T$ values per $U$ were chosen to fit a linear regression with the formula $R_T = a \cdot U$.

Slopes (a) were tested for parallelism using ANCOVA and then compared by means of a Two-Way-ANOVA.

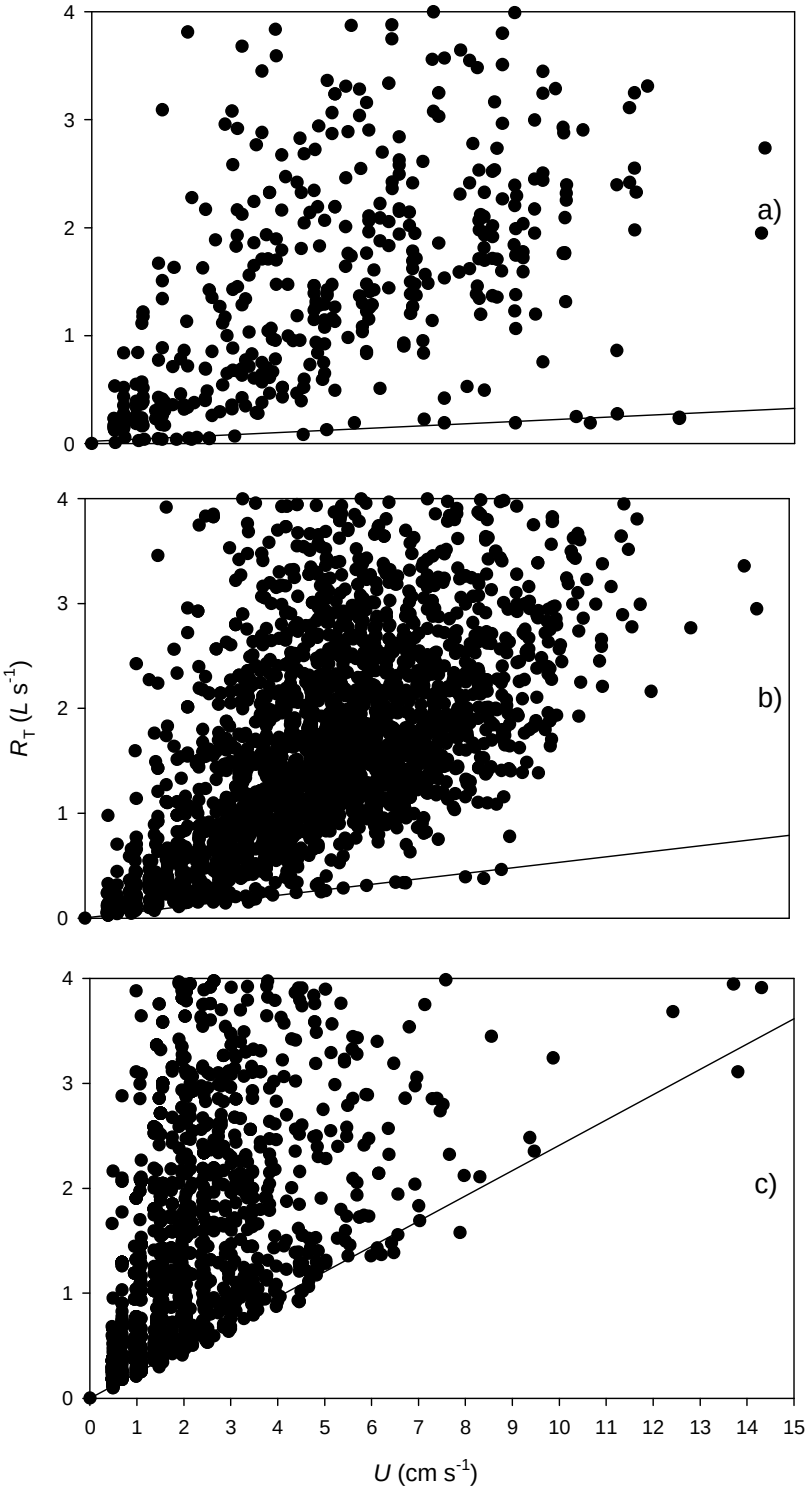


Figure 1: $R_T$ (bl) plotted against swimming speed $U$ (bl s$^{-1}$) in a) eel (anguilliform, 41.2 cm bl), b) sea bream (subcarangiform, 19.4 cm bl), c) striped surfperch (labriform, 18.4 cm bl). The correlation line fits the minimum $R_T$ and is described by $R_T = a \cdot U$.

## *Results*

When plotting $R_T$ of the centre of mass (cm) against $U$ (cm $s^{-1}$), the resulting lower limit of the minimum values of $R_T$ can be described by a line with the formula $R_T = a \cdot U$ (Fig 1). The values for a, *i.e.* the slope of the curve, maximum velocity reached and average velocity are given in table 1. Maximum and average velocity are not significantly different between the species (Two Way ANOVA, n=7). $U$ and the lowest $R_T$ values are significantly correlated (linear regression, n=7, $p<0.001$) for all cases. The slope of the resulting linear regression curve (a) is the lowest for eel, followed by seabream, then by small striped surfperch and large striped surfperch. The slopes are significantly different between the species (F-test, n=7, $p<0.001$) but there is no difference between large and small striped surfperch.

Table1: Maximum swimming speed, average swimming speed and the slope of the linear regression line of minimum turning radius plotted against swimming speed, described by the formula $R_T = a \cdot U$. $p<0.05$

| | *Eel* | *Seabream* | *Striped Surfperch* |
|---|---|---|---|
| *Maximum velocity* (cm $s^{-1}$) | 25.26 ± 15.12 | 27.49 ± 17.27 | 26.01 ± 17.89 |
| *Average velocity* (cm $s^{-1}$) | 4.76 ± 1.22 | 5.05 ± 1.96 | 4.52 ± 1.04 |
| *Slope (a)* | $0.0228 \pm 0.003^a$ | $0.0538 \pm 0.009^b$ | $0.2856 \pm 0.0997^c$ |

## *Discussion*

When plotting $R_T$ against $U$ the data points show a random distribution but with a lower limit. The lower limit for $R_T$ is positively correlated with speed and shows that the sharpness of possible curves is decreasing with increasing swimming speed. The resulting slope of this lower limit is increasing with decreasing use of the trunk region for swimming. The anguilliform eel, using the largest amount of its trunk region for propulsion, has the lowest lower limit and thus can perform sharp curves even at high swimming speeds, while the MPF swimming surfperch, using its pectoral fins for propulsion and not the trunk

musculature before gait transition (Drucker & Jensen, 1996) is more limited in the sharpness of turns at higher swimming speeds.

It has been shown that in determining turning performance the rigidity of the body and the mobility and position of the control surfaces is particularly important (e.g. fins, paddles, flippers; Webb, 1984; Weihs, 1993; Bandyopadhyay et al., 1997; Fish, 1997, 2002; Walker, 2000). The present study is in line with this statement, showing the slope of the minimum line for turning radii at a given swimming speed being the lowest for the flexible anguilliform swimmer and the highest for the rigid pectoral fin swimmer.

Webb (1984) and Videler (1993) stated that minimum turning radii are independent of speed. However, it is shown here that turning radii are dependent on speed as well as on the fish species.

Webb & Fairchild (2001) compared MPF with BCF swimmers in their manoeuvrability, defined as the minimum specific turning radius (Webb, 1983), at different speeds and came to the conclusion that MPF swimming at higher speeds led to a greater manoeuvrability, including factors such as turning, breaking or backwards swimming. However, there were sufficient exceptions to indicate that other factors were important. The results of the present study indicate that MPF swimmers show less manoeuvrability in terms of specific turning radii than BCF swimmers at low speeds. Rivera et al. (2006) found a similar relationship of minimum turning radii and swimming speeds in turning painted turtle. Manoeuvrability, defined as the ability to turn in a confined space using the length specific minimum radius of the turning path in body length as a measure of this performance (Norberg and Rayner, 1987; Webb, 1994), appears to be negatively correlated with body rigidness (Aleev, 1969; Fish, 1997, 1999; Blake et al., 1995; Domenici and Blake, 1997). The results of the present study are consistent with this hypothesis.

Gerstner (1999) found similar minimum $R_T$ in MPF as and BCF swimmers. The differences between the minimum $R_T$ of MPF and BCF swimmers found in the present study are due to the speeds they were performed at.

Both predators and prey benefit from high turning performance (Howland 1974). There is a linear relationship between turning radius and body length in aquatic vertebrates (e.g., Domenici and Blake 1993, Domenici et al. 2004). Body length is cubically proportional to body mass. Therefore, turning radius should scale with body mass to the power of 1/3. A log–log correlation of turning radius versus body mass for fish, sea lions, and cetaceans fits a slope of 0.37 (Blake and Chan 2006). This implies that manoeuvrability decreases with body length as minimum turning radii increase with body length (Domenici 2001). However, the present study could not show a significant difference in the slope of the $R_T/U$ plot between small and large surfperch. The reason might be that the difference becomes significant only at larger size differences.

It can be concluded that minimum turning radii are positively correlated with speed. The higher the body flexibility and trunk use for propulsion, the lower the minimum turning radius per speed.

## *Acknowledgements*

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***Chapter 6***

# *Pectoral Fin Beat Frequency Predicts Oxygen Consumption During Spontaneous Activity in a Labriform Swimming Fish (Embiotoca lateralis)*

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*Copeia; to be submitted*

## *Abstract*

The objective of this study was to identify associated kinematic variables to quantify oxygen consumption during spontaneous labrifom swimming. Kinematic variables (swimming speed, change of speed, turning angle, turning rate, turning radii and pectoral fin beat frequency) and oxygen consumption ($MO_2$) of spontaneous swimming in *Embiotoca lateralis* were measured in a circular arena respirometer using video tracking. The main variable influencing $MO_2$ appeared to be pectoral fin beat frequency ($r^2 = 0.80$). No significant relationship was found between swimming speed and pectoral fin beat frequency. Complementary to other methods within biotelemetry such as EMG it is suggested that such correlations of pectoral fin beat frequency may be used to measure the energy requirements of labriform fish such as *E. lateralis* in the field.

## *Introduction*

Locomotion represents a large component of the bioenergetic budget of many fish species (Koch and Wieser, 1983; Boisclair and Leggett, 1989; Boisclair and Sirois, 1993). Nevertheless, this component is poorly documented (Soofiani and Hawkins, 1985; Lucas et al., 1991; Ney, 1993). Locomotion can be described as steady and unsteady swimming. Steady swimming is defined as swimming in a straight line at a constant speed (Blake, 1983; Videler, 1993), and is mainly studied in swimming respirometers (Brett, 1964; Beamish, 1978; Korsmeyer et al., 2002). Unsteady swimming involves manoeuvres, acceleration and deceleration (Blake, 1983; Videler, 1993), commonly referred to as spontaneous swimming activity. While the kinematics of unsteady swimming has been studied extensively (Domenici and Blake, 1997; Blake, 1983; Videler, 1993), its energetic aspects are less well known (Webb, 1991; Tang and Boisclair, 1993; Krohn and Boisclair, 1994; Steinhausen, 2005).

Spontaneous activity includes common behaviours such as safeguarding territories, searching for food, avoiding predators and mating. The bioenergetics of spontaneous swimming activities are relevant to estimate the locomotion costs of free-ranging fish and are therefore are ecologically important.

Labriform swimming, *i.e.* using pectoral fins for lift-based propulsion at slow to moderate speeds (Webb, 1973; Drucker and Jensen, 1996a), is a widespread locomotion mode in structural complex habitats, where it is believed to provide greater manoeuvrability and stability at low speeds (Korsmeyer et al., 2002). Therefore, labriform swimming occurs in many groups of perciform fishes, including numerous families inhabiting coral reefs (Thorsen and Westneat, 2005). Nevertheless, to our knowledge, the energy consumption of spontaneous swimming activity of labriform fish has never been quantified. This is important because forced swimming models of steady activity are insufficient in explaining spontaneous activity (Tang et al., 2000).

The striped surfperch (*Embiotoca lateralis*) is an ideal species for studies of labriform swimming behaviour as they rely on pectoral fins for propulsion over a wide range of speeds (Drucker and Jensen, 1996a). The objective of the study was to investigate the energetic costs of spontaneous swimming in striped surfperch, quantified by a) speed ($U$), b) acceleration and deceleration ($A$), c) turning angle ($A_T$), d) turning radius ($R_T$) and e) the pectoral fin beat frequency ($f_P$) and to find a predictor that can be used as a tool to measure the energy expenditure in the field at very low to moderate swimming speeds.

## *Material and Methods*

### *- Fish -*

The study was carried out at Friday Harbor Marine Labaratories of the University of Washington, Friday Harbor, San Juan Island, Washington, USA. Striped surf perch *Embiotoca lateralis* were collected by beach seining at Jackson Beach on San Juan Island. Fish were maintained unfed for at least 72 h prior to experimentation in holding tanks in a constant flow through of seawater at a temperature of 13 ± 0.5 ºC.

### *- Respirometry of spontaneous swimming -*

Routine metabolic rates were measured in striped surf perch (body length, $L$ = 18.6 ± 0.4 cm, body mass, $M$ = 128 ± 0.2 g, n = 7) in a perspex circular arena respirometer (diameter 41.0 cm, height 11 cm, volume 14.2 l, Steinhausen, 2005, fig. 1) after being left to settle for minimum of 6 h. This period appeared to be sufficient for other fish to settle to a routine oxygen consumption rate (Steffensen et al., 1984; Jordan et al., 2001). The respirometer was submerged in water to keep the temperature constant at 13 ºC. Two external pumps were connected to the respirometer. One pump re-circulated the water continually at a slow flow rate (~0.7 l $min^{-1}$) to mix the water. Oxygen partial pressure was measured with the system closed using a sharp fibre optic sensor ($Pst_3$, Presens,

Germany) connected to an oxygen meter (Microx TX3; Presens, Regensburg, Germany). The second pump flushed the respirometer through two remaining ports prior to each measuring period. Each period was initiated by flushing the chamber with oxygenated water for 15 min, followed by a 1 min period to achieve a steady state in the chamber. Oxygen partial pressure ($pO_2$) was measured at a frequency of 1 Hz over the following 40 min before another cycle was started. Each trial consisted of 6 periods of fresh water recirculation and 4 measuring periods (Fig 2).

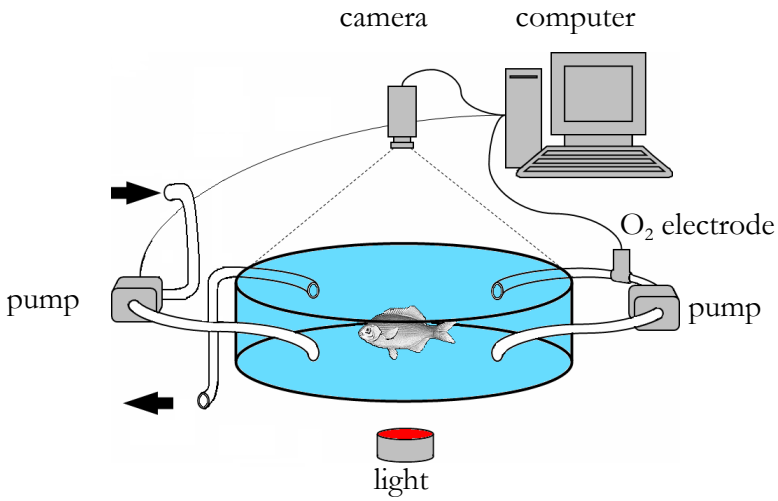


Fig. 1 Simplified illustration of the equipment used to determine the spontaneous swimming levels and routine metabolic rates in *Embiotoca lateralis*. During flush period (10 min) the respirometer exchanged with water from a surrounding tank and decline in $pO_2$ was measured over 40 min. The position of the fish was tracked with 5 Hz with a mounted CCD camera. The experiments were carried out under dim light conditions. See text for further details.

*- Video tracking and analysis -*

The fish were filmed at 25 fps with a CCD-camera (TVCCD 460; Monacor, Denmark) mounted at a height of 1 m above the respirometer. In order to synchronize the video of the swimming fish with the $MO_2$, the onset of each

measuring period was signalled by a brief flash of the infra red light source. Images were digitized by a video capture card (Pinnacle PCTV Rave) with a resolution of 640 x 480 pixels. Data were collected with Labtech Notebook Pro via a Measurement Computing PCMCIA-DAS16D/D interface board. An infra-red light source (Monacor IR-10) illuminated the fish from below. The geometrical centre of the resulting digitized silhouette of the fish was tracked as a *xy* coordinate pair at a frequency of 5 Hz using LoliTrack software (Loligo Systems, Denmark). A script aligning *xy* coordinates and the simultaneous decline in $pO_2$ ($r^2 = 0.84 \pm 0.11$) into periods of 10 min was written with Labtech Notebook Pro-software.

Pectoral fin beat frequency ($f_P$) was counted from movie files in AVI format collected during closed respirometry cycles. Each of the 24 periods per individual (6 hours times 4 periods) were ranked according to low, medium or high $MO_2$. From each category, three periods of each 10 minutes were randomly assigned for analysis of $f_P$. This resulted in 9 periods per individual, i.e. a total of 63. In one case, effect of $f_P$ on $MO_2$ was counted during the entire period of 6 hours (i.e. 4 periods times 6 hours).

*- Quantification of activity -*

Swimming speed (*U*) was calculated as the displacement of the geometrical centre of mass of the fish over time and expressed as average bl $s^{-1}$ over 10 min. For every consecutive frame, acceleration and deceleration (*A*) was calculated as the derivative of *U*, and expressed in absolute values because the positive and negative accelerations would have cancelled each other and would have resulted in average *A* close to 0. The turning angle ($A_T$) was calculated as the angle between two consecutive vectors characterising the direction of the fish in a horizontal plane, given by $\cos \theta_i = u_{i-1} * u_i (U_{i-1} * U_i)^{-1}$. The turning radius ($R_T$) was determined according to Domenici and Blake (1991) as the radius of the circle

that can be calculated from three consecutive positions of the centre of mass. $f_P$ was adjusted to a standard body mass of 0.1 kg according to

$$f_{P(0.1kg)} = f_P (M\ 0.1^{-1})^{(1-0.12)}$$

where $f_{P(0.1kg)}$ is pectoral fin beat frequency of a 0.1 kg fish, $M$ is the body mass in kg and 0.12 is a scaling exponent (Drucker and Jensen, 1996b).

*Calculation of oxygen consumption*

Mass specific oxygen consumption ($MO_2$) was calculated using the formula

$$MO_2 = a\ V_{resp}\ \beta\ M^{-1}$$

where $MO_2$ is the oxygen consumption (mg $O_2$ $kg^{-1}$ $h^{-1}$), $a$ is the slope of the linear regression ($\Delta O_{2\ sat}\ \Delta t^{-1}$), $V_{resp}$ is the volume of the respirometer minus the volume of the fish (l) with body mass considered equal to body volume (1kg = 1l), $\beta$ is oxygen solubility (mg $O_2$ $mmHg^{-1}$ $l^{-1}$) and $M$ is the body mass of the fish (kg). To correct for mass specific oxygen consumption, metabolic rates were adjusted to a standard body mass of 0.1 kg using the formula

$$MO_{2(0.1kg)} = MO_2 (M\ 0.1^{-1})^{(1-0.79)}$$

where $MO_{2\ 0.1kg}$ is the corrected consumption, $M$ is fish body mass and 0.79 is a scaling coefficient (Clarke and Johnston, 1999).

Oxygen consumption as a function of pectoral fin beat frequency ($f_P$) was described using a power function

$$MO_2 = a + b f_P^{\ c}$$

with $a$ being the estimate of the standard metabolic rate (SMR), *i.e.* the $MO_2$ at zero activity, and $b$ and $c$ being constants.

*Statistics*

The combined influence of all variables ($U$, $A$, $A_T$, $R_T$, and $f_P$) on $MO_2$ was explored using forward stepwise regression ($p<0.05$, n = 7, STATISTICA 6.0,

StatSoft, Inc., 2001). $MO_2$ was log transformed ($\log MO_2$) for linear regression analysis.

## *Results*

*- Spontaneous swimming activity over time -*

Figure 2 shows an example of a correlation of pectoral fin beat frequency ($f_P$) with oxygen uptake of an individual over the period of 5 hours and 40 minutes. No measurements were taken for 30 minutes when the respirometer system was flushed after a measuring period of 40 minutes.

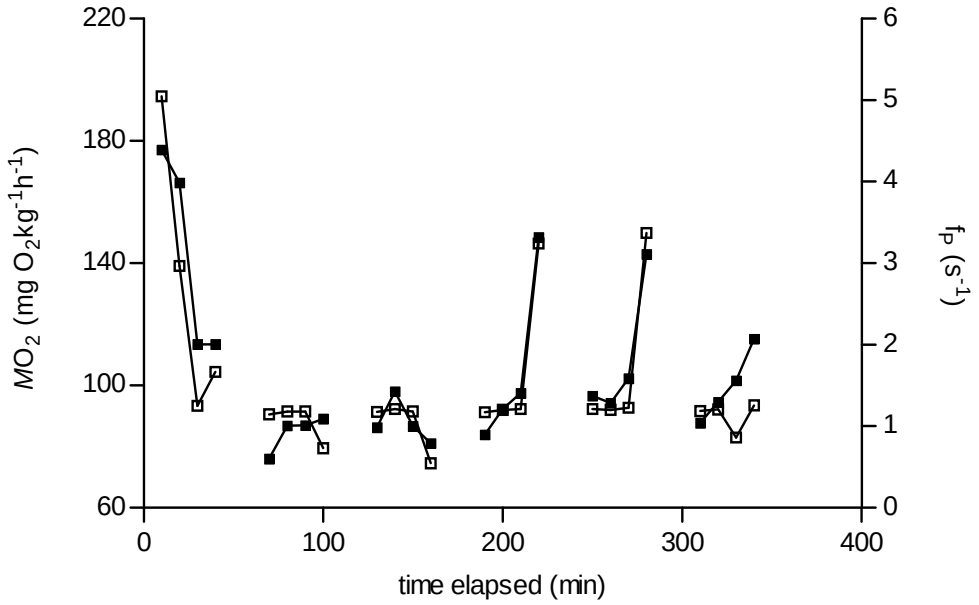


Fig. 2. Simultaneous recording of oxygen consumption (*MO2*) and fin beat frequency (*Pf*) of a single surfperch at spontaneous swimming. Each symbol represents a 10 min average.

*- Oxygen uptake and kinematic variables modelling g-*

$MO_2$ in striped surfperch in the circular arena respirometer ranged between 72 and 334 mg kg$^{-1}$ h$^{-1}$. The mean swimming speeds were 0.21 ± 0.1 bl s$^{-1}$ and ranged between 0.16 and 0.38 bl s$^{-1}$ (n=7). A multilinear regression model for log-transformed $MO_2$ was computed:

$$\log MO_2 = 1.59 + 0.95\ U + 0.003\ A + 0.001\ A_T + 0.003\ R_T + 0.047\ f_P$$

From analysis only $f_P$ and $A_T$ were significantly contributing to log $MO_2$ with an $r^2$ of 0.66 and 0.32, respectively (forward stepwise regression, $r^2 = 0.76$, $p < 0.05$). Plotting $MO_2$ against $f_P$ (fig. 3) resulted in an exponential curve described by the formula

$$MO_2 = SMR + b\, f_P{}^c$$

with SMR being 85.93 ± 11.59 mg $kg^{-1}$ $h^{-1}$, $b$ being 17.37 ± 9.18 and c being 1.08 ± 0.25 ($p < 0.05$, $r^2 = 0.71$).

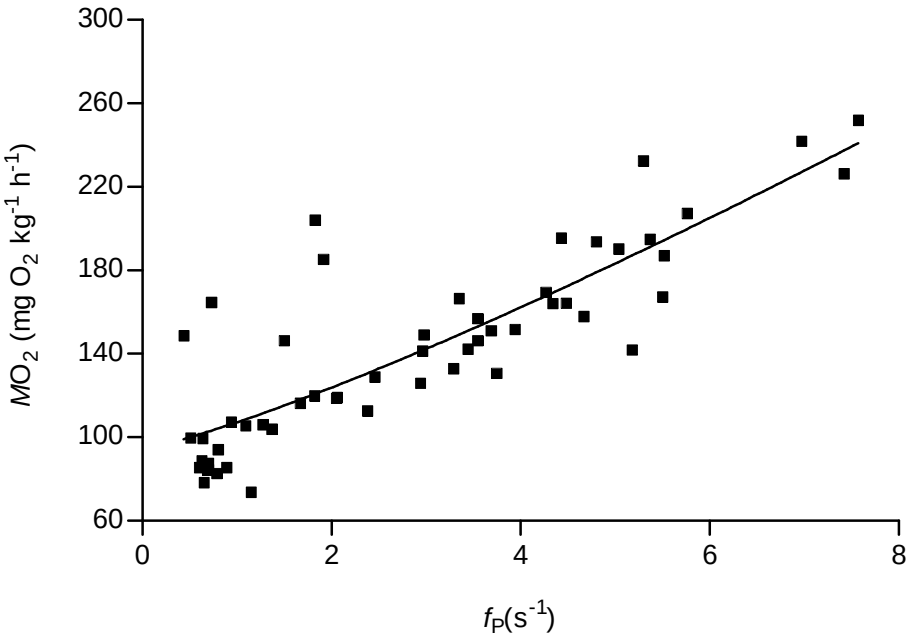


Fig. 3. Relationship of pectoral fin beat frequency ($Pf$) on log $MO_2$ at spontaneous swimming ($MO_2 = SMR + b\, f_P{}^c$, $r^2 = 0.80$).

## *Discussion*

The results suggest that energy requirements of a labriform fish during spontaneous swimming can be accurately predicted ($r^2 = 0.71$) using the pectoral fin beat frequency. Also, future studies of metabolic rates and activity of free labriform swimming fish may benefit from including techniques that allow direct measurements of $f_P$ in the field. For example, a number of laboratory and field studies have applied electromyography EMG to correlate muscular recruitment with axial swimming kinematics (Jayne and Lauder, 1995a; Jayne and Lauder,

1995b) and to determine swimming costs of axial swimmers (Hinch and Rand, 1998; Standen et al., 2002). Cooke et al. (2004) predicted that some of the most interesting future findings in ecology will be derived from studies involving biotelemetry (*i.e.* remote measurement of physiology, behaviour and/or energetics). Given our results, we suggest that EMG records of the pectoral activity may be used to measure fin beat frequency (rather than speed and distance) and hence to estimate oxygen consumption in striped surf perch and other labriform fishes. Correct insertion of electrodes into the pectoral musculature may pose a challenge. However, Drucker and Jensen (1997) provided detailed information upon the timing and intensity of the pectoral fin musculature during forced swimming using this technique in surfperch. Previously, radio telemetry of transmitting EMG signals in sea water was complicated since radio signals are rapidly attenuated, but Dewar et al. (1999) developed an acoustic tag for monitoring EMG in four free swimming marine species. Overcoming the challenges mentioned above may lead to future activity estimates of free-ranging labriform fish.

The minimum metabolic rates found by extrapolating $f_P$ to zero activity (fig. 3) resulted in values of 85.93 ± 11.59 mg $O_2$ $kg^{-1}$ $h^{-1}$. These values closely resemble the results by Cannas et al. (2006) reporting an SMR of 82 mg $O_2$ $kg^{-1}$ $h^{-1}$ (adjusted to a body mass of 0.1 kg) for striped surf perch in a conventional swimming respirometer. Thus, the similarity among the SMR estimates suggests a sufficient accuracy of the oxygen consumption measurements in the circular arena respirometer.

Commonly, $MO_2$ is measured in swimming respirometers at high swimming speeds. Work on forced linear swimming may be particularly relevant for pelagic fish that show long periods of relatively steady swimming in nature. However, various authors have shown that the costs of locomotion during spontaneous swimming in non-pelagic fish species are higher than that of forced swimming (Weatherly and Gill, 1987; Webb, 1991; Steinhausen, 2005). This is most likely

due to the additional resistance components of the spontaneous swimming (Webb, 1991). Relatively low speed swimming (spontaneous) as it occurs in nature may imply a high degree of manoeuvring, stability control and accelerations/decelerations with loss of momentum, particularly in fish that live in structurally complex environments (Domenici, 2003). This may in particular apply to labriform swimmers, such as Embiotocidae, which rely on pectoral fin activity for all types of locomotion as well as a synchrony with their ventilation rates (Webb, 1975). As a result, the relationship between speed and $\dot{M}O_2$ during such activity patterns may be weak. This implies that the use of pectoral fins in striped surf perch in our experiment is mainly related to behaviours other than forward locomotion, including manoeuvring, stability control and hovering.

The observed low swimming speeds in the circular arena respirometer ($0.21 \pm 0.1$ bl $s^{-1}$) may be due to a number of factors. Considering a potential effect of laboratory confinement, it is possible that our results may be influenced by the artificially confined fish. The tank was relatively small (tank diameter $\approx$ 2.2 bl) due to restrictions imposed by respirometry techniques. Space availability favours higher speeds (Tang and Boisclair, 1993). Another explanation of the low speeds observed during spontaneous swimming may be the social behaviour of striped surf perch. Grouping behaviour may have discouraged the solitary fish to swim actively within the arena respirometer, compared to fish swimming in a group. This is in agreement with previous studies on cyprinid fishes showing social facilitation (reviewed by Smith, 1991). It remains to be established if the preferred swimming speed and perhaps swimming kinematics are influenced by the presence of conspecifics.

In conclusion these first results on the metabolic costs of spontaneous activity in a labriform fish shows that $f_P$ can be used as predictor for swimming energetics, and in combination with EMG records it can provide a good tool for measurements of $\dot{M}O_2$ in the field.

***Acknowledgements***

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***Chapter 7***

# *Ammonia Exposure affects Fast Start Performance and Predation Behaviour in Brown Trout (Salmo trutta L.)*

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## *Abstract*

In fish, fast starts are used for escape and predation and are therefore an ecologically important movement. Fast starts are generated by glycolytical muscle performance and are influenced by many internal and external factors. It is known that ammonia pollution has a major effect on the glycolytical muscle action, thus creating conditions in which fast start performance might be reduced and predation rates altered. Therefore, escape response and predation strikes were investigated in brown trout (*Salmo trutta*) of 10 and 20 cm body length exposed to an elevated (1 $mg.1^{-1}$) ammonia concentration for 24 and 96 hours. Various locomotor and behavioural variables were measured.

In C-starts, ammonia exposure had no effect on response latency. After 96 hours of exposure, cumulative distance, maximum swimming speed and turning radius were all significantly reduced and the direction of escape became at random. Predation strikes were also affected. Distance, speed and turning radius were significantly lower in exposed fish. Predator behaviour was also altered and the number of prey captured was reduced. The effect of ammonia exposure was more pronounced in large fish than in small fish.

This study shows that ammonia exposure affects brown trout escape response mainly through a reduction in fast start velocity and through an impairment of directionality. Thus, in addition to a reduced strength of the response, ammonia exposure may also reduce the fish's elusiveness facing a predator. Predation rate and social interactions are disrupted and predator prey relationships may be altered.

## *Introduction*

Fast starts are highly energetic swimming bursts, started either from rest or imposed upon periods of steady swimming (Jayne and Lauder, 1993, Domenici and Batty, 1994). They are an ecologically important movement in fish since they are used for escaping predators and prey capture. Kinematically, different types of fast starts can be distinguished: 1) C-starts, which are generally used for escape responses, where a fish takes a C-like body shape before bursting, resulting from a contraction of the lateral musculature on the opposite side relative to the stimulus, and 2) S-starts which are used for escape but also predation, where an S-like shape can be observed, resulting from simultaneous contraction from the musculature on both sides (Domenici and Blake, 1997).

Predator prey relationships are shown to be influenced by kinematics. Dill (1974) demonstrated that the prey's reactive distance increases with the speed and depth of the predator's body profile. A rapidly approaching predator may trigger an early response in the prey. Therefore, fast start speed of attacking predators is often sub-maximal (Webb, 1984, Harper and Blake, 1991). Also, predators aim at the centre of mass of their prey, perhaps because the centre of mass is the best target, since it can be located from the prey's geometry and it also is the point of a prey that moves the least during escapes (Webb and Skadsen, 1980). Domenici and Blake (1991) and Eaton and Emberley (1991) showed that turning angles in escape responses are variable but are mostly directed away from the attacking predator (Domenici and Blake, 1997). Striking angles of the predator may be species specific (Hoogland et al., 1956) and are possibly influenced by gape limitation in addition to the prey's shape and other morphological factors, such as false eye spots (Domenici and Blake, 1997).

Fast starts are generated by white glycolytical muscles and are thus a movement of high but short muscle performance. White muscles are known to be very susceptible to ammonia toxicity and it has been shown that ammonia exposure decreases swimming performance in fish (Beaumont et al., 1995, Shingles, et al.,

2001). Ammonia gas can diffuse into fish across the gills, and therefore water $NH_3$ concentration determines the potential for toxicity (Shingles et al., 2001). The toxic effect of elevated ammonia concentrations in the water is attributed to a reduced outward diffusion of ammonia through the gills, and even a reversed inward ammonia flux can occur. As a result, ammonia levels in the fish plasma increase. Another possible mechanism for plasma ammonia build-up at high external ammonia levels is the decreased ammonium outward flux through the $Na/NH_4^+$ exchanger (Wood, 1992). Beaumont et al. (2000a) suggested that increased $NH_4^+$ levels alter the metabolic status within the fish, arising from the effects on a number of metabolic pathways and may lead to premature muscle fatigue and, hence, a reduction in swimming performance. Also, measuring the resting membrane potential of white muscle Beaumont et al. (2000b) found a significant partial depolarisation. This was consistent with a predicted depolarisation, based on the measured distribution of ammonia between intracellular and extracellular compartments, and was suggested to be due to the displacement of $K^+$ by $NH_4^+$. As ammonium ions are an allosteric activator of phosphofructokinase (Su and Storey, 1994) and inhibit pyruvate carboxylase (Zaleski and Bryla, 1977), elevated ammonia levels may increase the rate of flux through the glycolytic pathway, depleting stored glycogen levels and possibly also disrupting its regeneration in white muscles (Beaumont et al., 1995). High ammonia concentrations might, therefore, reduce fast start performance by impairing anaerobic capacity. Salmonids are known to be susceptible to even low ammonia concentrations in freshwater (Shingles et al, 2001).

The objectives of this study were to evaluate the effects of ammonia exposure on physiological and ecological aspects of escape and predation in brown trout (*S. trutta*). An integrative approach was used in order to test the hypothesis that ammonia exposure has an effect on timing and locomotor variables of escape performance and predation fast start. Also, to investigate if such an effect could affect predator prey interactions, predator behaviour and prey capture rate were

considered. The concentration of 1 mg $l^{-1}$ used in this study is the legal threshold concentration for Flemish surface water.

## *Material and Methods*

### *- Fish -*

Brown trout of a body length (*L*) of 10 cm (10.01 ± 0.09 cm, hereafter referred to as 'small') and 20 cm (20.16 ± 0.27 cm, hereafter referred to as 'large') were obtained from a fish farm and transported to the University of Antwerp were they were held in 200 litre tanks in groups of 15 large or ca 100 small individuals in softened Antwerp City tap water at a constant temperature of 15.0 ± 0.4°C for at least four weeks before experiments started. Tanks were in flow-through with a turn over rate of 100 litres per day. Additional filtering occurred by means of a triple filter consisting of cotton, active carbon and lava stone. Fish were fed with Nutra Fish Food (Skretting, France) once a day. 72 hours before the experiment started, fish were transferred to a 200 litre flow through tank in groups of two individuals where they were starved.

### *- Exposure -*

The 96 h ammonia exposure started in the 200 litre flow-through tank where groups of two fish were kept for 72 h before the experiment. The volume of 200 litres was spiked with the required amount of an $NH_4Cl$ stock solution (Merck, Darmstadt, Germany), and subsequently ammonia solution was added by means of a peristaltic pump (Watson-Marlow, Falmouth, UK) in order to compensate for the water renewal. This resulted in a constant concentration of 1.09 ± 0.12 mg/l. For the 24 h exposure experiment, and for the last 24 hours of the 96 h exposure experiment, exposure took place in the experimental tank. For the exposure experiments, the water was spiked with ammonia solution and reached concentrations of 1.02 ± 0.10 mg/l. The control groups were not exposed to ammonia.

During the experiments the fish remained in the same pairs as during the preceding 72 h.

A second set of fish was exposed in the flow-through tanks as described above, and after 24 and 96 hours of exposure fish were netted and quickly killed in an overdose of buffered MS-222 (1 $gl^{-1}$, pH 7.4, Acros Organics, Geel, Belgium). Fish were immediately put on ice and a blood sample was drawn from the caudal blood vessel. Blood was immediately centrifuged for 3 min in 1.5mL bullet tubes (13200×g) and plasma was snap-frozen in liquid nitrogen. In the mean time, a small piece of white muscle was dissected and snap frozen in liquid nitrogen as well. Samples were later stored at −80 ◦C for ammonia analysis.

*- Experimental set up -*

24 h before the experiments started, two large fish were transferred to a square tank of 120 by 120 cm and two small fish were transferred to a round tank with a diameter of 95 cm. The water depth was 20 cm in both tanks and the water had a temperature of 15.0 ± 0.4° C. A black curtain prevented the fish from being disturbed.

A high-speed camera (Redlake Imaging Motion Scope PCI 1000 S) was used to record the fast starts at 500 Hz. The camera was positioned 2.1 m above the experimental tank. Video recording started ca 1 s before the stimulus and lasted 3 s. Observation of the fish behaviour in the experimental tank suggested that presence of another fish increased the activity level. Therefore, the experiment was always conducted with two fish. They were transferred to the experimental tank 24 hours before the experiment was conducted.

Escape responses were elicited by triggering the fall of a dummy (black PVC cylinder with a tapered tip, 2.8 cm diameter, 11.0 cm length) held 1 m above the experimental tank by an automatic release construction. In order to provide a sudden stimulus and allow calculation of the response timing, the dummy passed through a black tube (110 x 4 cm), suspended from above with its lower edge 5

mm above the water surface so that it was only noticed by the fish when it touched the water surface. In order to determine the timing of the impact into the water, preliminary tests were carried out by dropping the dummy above a mirror. The test showed that the propagating wave resulting from the dummy touching the water surface was visible outside the tube 2 ms after impact. Therefore, it was taken into account that the stimulus ($t_0$) occurred 2 ms before the wave was visible. Fish were tested in sets of two, of which only one individual was chosen for further analysis to avoid pseudoreplication. Therefore, twenty fish were used in total, while only ten were used for analysis. The fish being analysed was chosen as being the nearest to the triggering device, at a distance of approximately 5 to 20 cm, at the time of the startling. The distance of the large individual fishes to each other varied from approximately 15 cm to 120 cm and of the small fishes from approximately 10 to 70 cm.

Predation fast starts were triggered by means of life bait. A thin string (2.2 m long) was attached to a small carp's (L 3.4 ± 0.2 cm) abdomen behind the pectoral fins and the string was fixed at a height of 2.1 m so that the carp was swimming in small (ca 30 to 40 cm diameter) circles under the camera. Two large trout were introduced and feeding events were recorded with a high speed camera (see above). Again, fish were tested in sets of two, of which only one individual was chosen for further analysis to avoid pseudoreplication.

Behavioural observations were conducted using a PAL video camera (Sony Corporation DCR-HC39E) positioned above the tank at a height of 2.1m. Five small carp were introduced and the behaviour of two large trout was recorded for six consecutive hours. In total, 20 trout were used and 50 carps, i.e. ten replicates with two trout and five carps per replica.

*- Analysis -*

Water ammonia levels were determined using the salicylate-hypochlorite method according to Verdouw et al. (1978). Total muscle ammonia was extracted

according to the method described by Wright et al. (1995). Total muscle and plasma ammonia was then measured using an enzymatic kit (r-Biopharm 11 112 732 035 Boehringer Mannheim Darmstadt, Germany).

The centre of mass (CoM) of the trout was determined by hanging dead frozen fish from two different points (in front of the dorsal fin and at the cloaca) and determining the crossing point of the vertical extension of the two lines. Eight brown trout of each size class were used and the position of the CoM was determined to be at 0.45 ± 0.01 L from the tip of the head for the large fish and at 0.40 ± 0.04 L for small fish.

Measurements of orientation and locomotor variables were made following the methodology described by Domenici and Blake (1991, 1993). The XY coordinates of the centre of mass (CoM) and the tip of the head were digitised for each fast start sequence from video sequences (AVI files) exported on into Vernier logger pro 3.4.6 (Vernier Software & Technology, Texas) to perform a manual tracking of the fish's movements. Locomotor variables were calculated using the digitised coordinates, on which a five points smoothing polynomial regression procedure was applied for each derivative step (Lanzos, 1956) and the following parameters were assessed:

(1) Response latency: time interval between stimulus onset ($t_0$ as defined above) and first detectable movement of the escape response ($t_1$).

(3) Stage 1 duration: time interval between the moment of $t_1$ and the change in direction of the head ($t_2$); stage 2 duration: beginning at $t_2$ and ended when a further reversal of head turning direction occurred (Domenici & Blake 1997); total duration: Stage 1 plus stage 2 duration.

(4) directionality: fast starts were divided into 'away' and 'towards' responses on the basis of $t_1$ being oriented away or towards the stimulus, respectively.

(5) Initial orientation angle ($A_0$): angle between the line passing through the stimulus position and CoM ($D_0$) and the line passing through CoM and the tip of the head of the fish at the onset of the escape response $t_0$ ($Ds_0$, Fig. 1).

(6) Stage 1 angle ($A_1$): angle between $Ds_0$ and the line passing through the CoM and the head of the fish at the end of the stage 1 ($Ds_1$, Fig. 1).

(7) Escape trajectory ($A_2$): angle between $D_0$ and the line passing through the CoM and the head of the fish at the end of the stage 2 ($Ds_2$, Fig. 1). Both $A_0$ and $A_2$ were transformed, so that stimulus position was always considered to be on the right side of the fish, thus $A_0$ ranged from 0 to 180 and $A_2$ from 0 to 360° (Domenici & Blake, 1993a; Domenici, 2002).

(8) response types: a) single bend (SB) and b) double bend (DB). In DB responses, both stage 1 and stage 2 occur. For SB responses, stage 2 does not take place, since at the end of stage 1, fish straighten and glide

(9) Average turning rate: $A_1$ divided by the time taken to complete stage 1. The instantaneous turning rate was determined as the difference between the angles of two consecutive frames divided by the corresponding time interval (i.e. 2 ms).

(10) Minimum turning radius: smallest radius of the approximately circular path of the CoM during an escape fast start.

(11) Distance time variables: a) total distance ($D_{tot}$), b) maximum speed ($U_{max}$) and c) maximum acceleration ($A_{max}$) were conducted over the mean fast start duration of all treatments (stage 1 plus stage 2 durations). c) As calculation of distance-related variables within a given time was suggested by previous authors (Webb, 1976; Domenici & Blake, 1993) to avoid any performance bias due to differences in fast-start duration, $D_{100}$, the distance covered by each fish within 100 ms after the stimulus onset, considering both response latency and cumulative distance, was used (Lefrancois et al. 2005).

For the analysis of escape performance all the above were determined while the analysis of the predation fast starts included the following variables: maximum speed ($U_{max}$), maximum acceleration ($A_{max}$), fast start duration, total distance ($D_{tot}$), and the distance covered by each fish within 100 ms after the first movement was detectable that led to a predation fast start, $D_{100}$.

For behavioural observations the following predation related variables was measured: (1) prey handling (time spent feeding), (2) predation (time period from moving towards the prey until ingestion of the prey), (3) success ratio (number of feeding events/number of predation attempts), (4) predation rate (number of feeding events/observation time) and (5) number of prey captured. The non-predation related variables measured were: (1) agonistic behaviour (time period of intraspecific aggressive behaviour), (2) resting (time spent immobile) and swimming (time spent moving freely in the tank). The dominant individual was determined by its aggressive behaviour against the subordinate individual.

The experiment was replicated 10 times for both control and ammonia exposed fish.

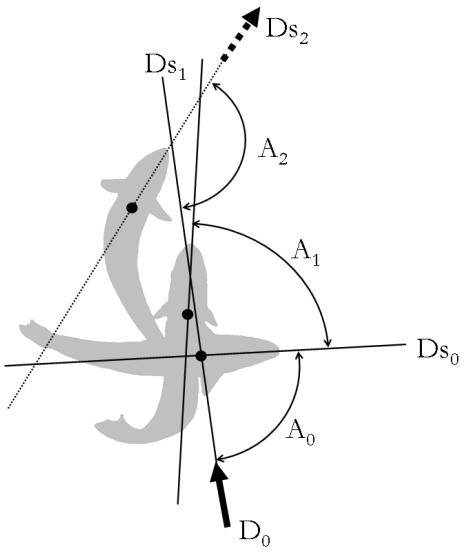


Fig 1: Illustration of the angular variables (solid arrow: stimulus direction; dotted arrow: escape direction). $D_0$, line passing through the stimulus position and the fish centre of mass (CoM); $D_{S0}$, line passing through the CoM and the head of the fish at the onset of the escape response; $D_{S1}$, line passing through the CoM and the head of the fish at the end of the stage 1; $D_{S2}$, line passing through the CoM and the head of the fish at the end of the stage 2; $A_0$, initial orientation; $A_1$, stage 1 angle; $A_2$, escape trajectory

*- Statistics -*

Escape response: To determine if ammonia exposure had an effect on directionality of response (i.e., 'away : towards'), a two tailed binominal test was

performed. Circular statistics (Mardia-Watson-Wheeler test, *W*-test) was used to assess the effect of ammonia exposure on $A_2$. The effect of ammonia exposure on the locomotor performance variables ($U_{max}$, $A_{max}$, duration, $D_{tot}$ and $D_{100}$) and the directional variables was analysed using ANOVA with a Bonferroni post-hoc test. The significance level for all tests was determined at $p<0.05$. N was 10 for all tests, i.e. the total number of trout was 20, with only one fish per set of two used for analysis.

Predation fast starts: The effect of ammonia exposure on the locomotor performance variables ($U_{max}$, $A_{max}$, duration, $D_{tot}$ and $D_{100}$) was analysed using ANOVA with a Bonferroni post-hoc test. The significance level for all tests was determined at $p<0.05$. N was 10 for all tests, i.e. the total number of trout was 20 with only one fish per set of two used for analysis.

Behavioural observations: The effect of ammonia exposure on behavioural traits, numeral and temporal parameters, was analysed using ANOVA with a Bonferroni post-hoc test.

For each treatment a total of 20 fish was used of which half were considered dominant (N = 10) and half were subordinate (N = 10).

All experiments complied with the regulations of the Ethical Board of the University of Antwerp

## ***Results***

*- Escape performance -*

Results of C-start kinematics analysis show that in small (10 cm) fish maximum velocity ($U_{max}$) significantly decreased after 96 hours of ammonia exposure as it did in large fish (Fig 2a, ANOVA, $p<0.05$, N = 10). Maximum acceleration ($A_{max}$) also shows a significant difference between the treatments but without a difference between the size classes (Fig. 2b, ANOVA, $p<0.05$, N = 10).

Total duration of C-starts increased in both size classes after 96 hours of exposure and differed between the size classes. Here, only stage two contributed

significantly to the differences (Fig. 2c, ANOVA, $p<0.05$, N = 10). Total distance ($D_{tot}$) and distance over 100 ms ($D_{100}$) show similar effects results. $D_{tot}$ in C-starts differed significantly between 0 and 96 hours of exposure as did $D_{100}$ in both, small and large fish (Fig. 2d, ANOVA, $p<0.05$, N = 10). Also, there was a size effect on $D_{tot}$ and $D_{100}$ with higher values in large fish (ANOVA, $p<0.05$, N = 10). No agonistic behaviour was observed between the small individuals.

Directionality analysis in small fish revealed an "away : towards" response ratio of stage 1 that was only different from random (i.e. 50% away : 50% towards) in small fish before exposure (90 ± 31.62 % away) and after 24 hours of exposure (70 ± 48.30 % away) and in large fish only before exposure to ammonia (80 ± 42.16 % away; binominal test, $p<0.05$).

There was no variation of the angle between stimulus and initial orientation between the conditions (angular mean ± angular deviation, W-test, $p>0.05$, N = 10, Lefrancois, et al., 2005).

After exposing small fish to ammonia for 96 hours, the escape trajectories went in no definite direction while controls and exposure for 24 hours revealed trajectories of 166.82 ± 43.99° and 169.24 ± 51.89°, respectively (angular mean ± angular deviation, W-test, $p<0.05$, N = 10, Lefrancois, et al., 2005). Large fish showed defined escape trajectories only before exposure, with a value of 171.12 ± 37.70° (W-test, $p<0.05$, N = 10).

Average turning rate in small fish after 96 hours of exposure (2.46 ± 0.25° $ms^{-1}$) differed significantly from control (3.21 ± 0.10° $ms^{-1}$, ANOVA, $p<0.05$, N = 10) as did minimum turning radius after 96 hours of exposure (1.99 ± 0.05 cm) from control (1.84 ± 0.05 cm, ANOVA, $p<0.05$, N = 10). In large fish, average turning rate was significantly lower after 96 hours of exposure (1.99 ± 0.14°$ms^{-1}$) compared to control (3.21 ± 0.10° $ms^{-1}$, ANOVA, $p<0.05$, N = 10, fig. 2e).

The minimum turning radius was increased after 96 hours of exposure in both, small fish and large fish compared to control (ANOVA, $p<0.05$, N = 10).

There was no significant variation of the angle between stimulus and initial orientation.

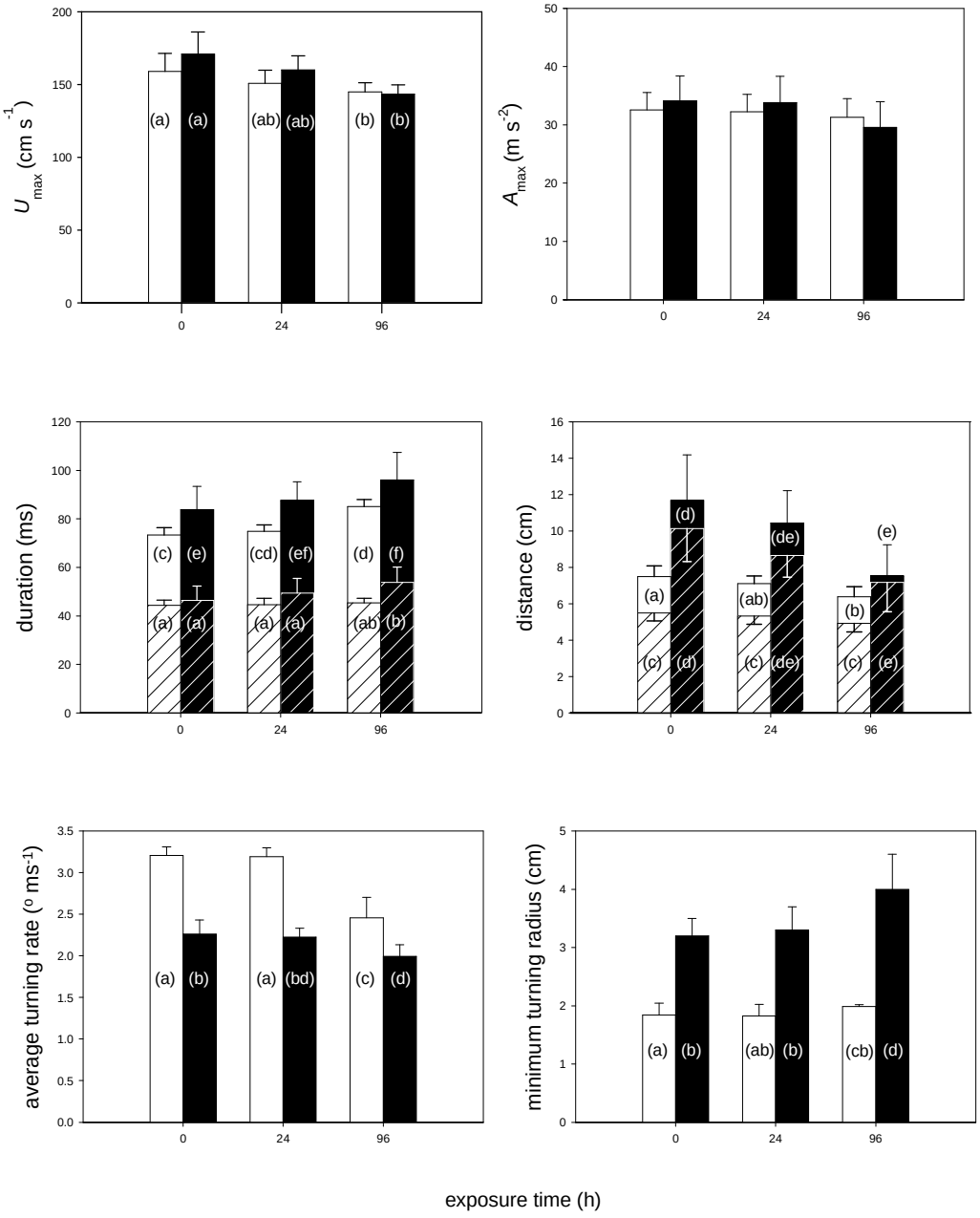


Fig 2: Kinematic data of escape performance in small (white) and large (black) brown trout. a) Maximum velocity ($U_{max}$), b) acceleration ($A_{max}$), c) of C-starts with stage 1 (striped) and stage 2 (plain), d) total distance (whole bar) and distance over 100 ms (striped), e) average turning rate in small and f) minimum turning radius after no exposure, 24 hours and 96 hours of exposure. Values are mean ± SD. Difference was significant at $p<0.05$.

*- Predation fast start -*

Both trout remained stationary, mostly at a side of the tank when the carp was introduced to the set up. The carp showed distress upon introduction but calmed down after a few minutes. For its mobility was limited it turned in circles of approximately 30 to 40 cm. Approximately 30 min after introduction, one trout approached with an average velocity of 0.94 ± 1.2 $Ls^{-1}$. Carp attempted to escape when the approaching trout reached a distance of 21.24 ± 9.33 cm. Few seconds before burst swimming was noted, trout approached the carp by coasting. Bursts consisted of severe beats with the tail fin, resulting in massive acceleration, and indicating the beginning of the predation fast start. The type of the fast start was similar in all cases with maximum velocity and acceleration reached at or slightly before the trout reached the position of the prey. The number of severe tail beats differed between 2 and 4, depending on the total distance of the fast start. Kinematic variables of trout predation starts showed significant differences between treatments. Maximum velocity was significantly reduced after 96 hours of exposure, and so was maximum acceleration (Fig. 5a). Total duration was significantly increased after 96 hours of exposure (Fig. 5b). Also the distance over 100 ms was reduced after 96 hour of exposure (Fig. 5c). There were no differences in the kinematic variables of the carp escape response between ammonia treatments (general values, mean ± sd: $U_{max}$: 0.51 ± 0.08 $ms^{-1}$, $A_{max}$: 19.21 ± 2.90 $ms^{-2}$).

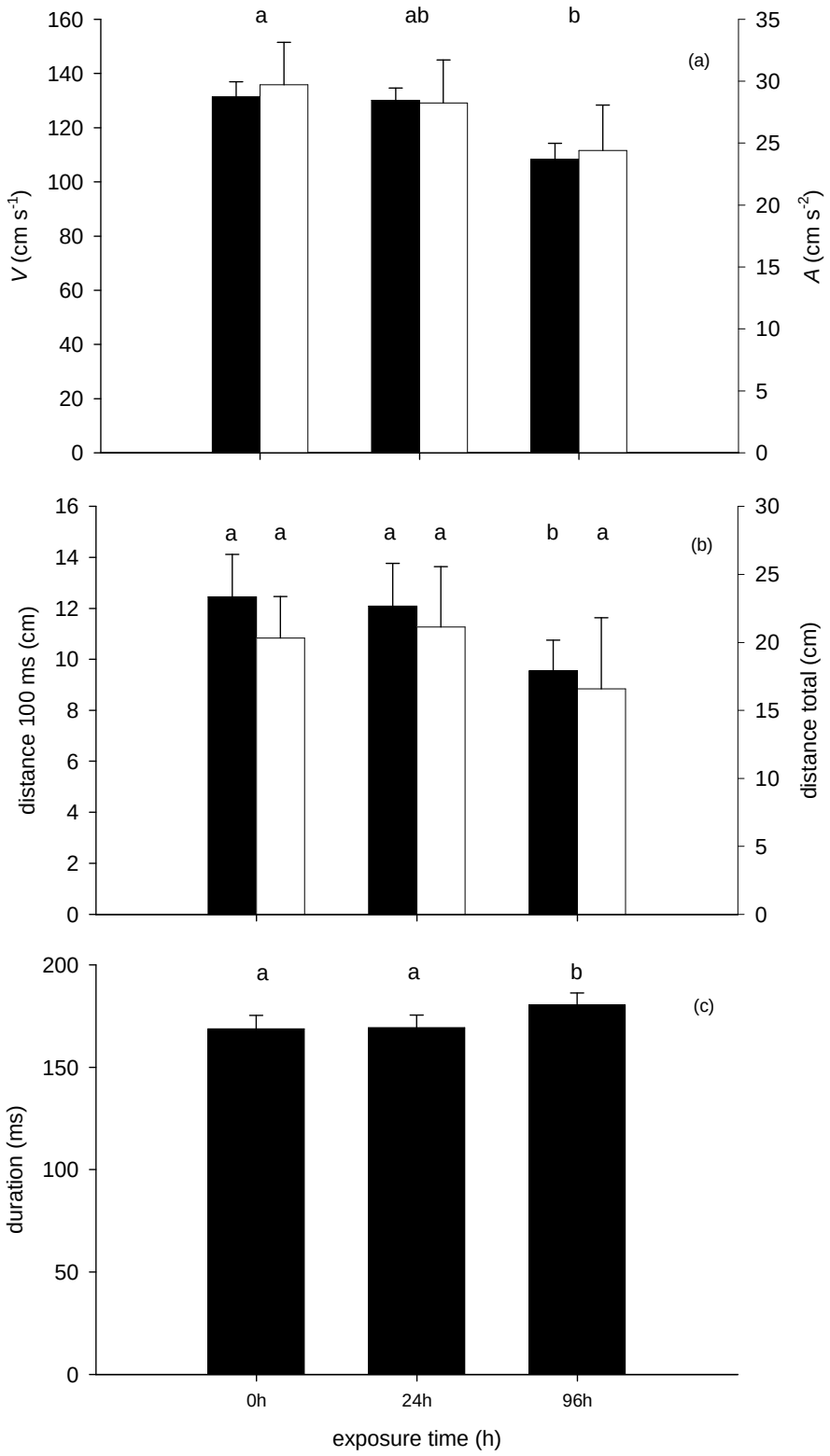


Fig 3: Kinematic data of predation fast start in large brown trout. a) Maximum velocity ($U_{max}$, black) and acceleration ($A_{max}$, white), b) total distance (white) and distance over 100 ms (black) and c) duration after no exposure, 24 hours and 96 hours of exposure. Values are mean ± SD. Difference was significant at $p<0.05$.

*- Behavioural observations of predator-prey interactions -*

The results of the observations are given in table 1. In general, the behaviour of the dominant individual was altered after 96 hours of exposure: the time spent resting as well as the time devoted to agonistic traits by the dominant towards the submissive individual were increased and prey capture rate and success were decreased.

*- Plasma and tissue ammonia -*

Plasma and muscle ammonia were significantly elevated in small trout exposed for 96h and in large trout exposed for 24h and 96h to ammonia (Table 2).

## ***Discussion***

The present study shows the effects of increased ammonia concentration on escape and predation performance in brown trout. The main findings of this study are: a) escape performance in small and large brown trout is significantly reduced after exposure to ammonia (fig 1, 2); b) predation fast start performance is reduced in large fish (fig 2) and c) predator prey interactions are impaired and predation rate is reduced after ammonia exposure (table 1). These findings show that an increased ammonia concentration in freshwater can significantly change predator prey interactions in piscivorous communities.

*- Escape performance -*

Brown trout of 10 cm *L* in clean fresh water at 15°C reached in C-starts a maximum velocity ($U_{max}$) of 159.0 cm $s^{-1}$, a maximum acceleration ($A_{max}$) of 32.6 m $s^{-2}$ and a total duration ($D_{tot}$) of 73.4 ms. In a study performed by Webb (1976) at 15°C in clean freshwater, rainbow trout (*Oncorhynchuss mykiss*) of 9.6 cm body length reached $U_{max}$ of 152.6 cm $s^{-1}$, $A_{max}$ of 33.2 m $s^{-2}$ and $D_{tot}$ of 71 ms, which is comparable to the values found in the small fish in the present study. Also, values on fish of 20.4 cm ($U_{max}$ = 167.2 cm $s^{-1}$, $A_{max}$ = 32.3 m $s^{-2}$, $D_{tot}$ = 78 ms, Webb, 1976) were similar to those obtained by large fish in the present study

Table 1: Behavioural traits of a dominant and a subordinate individual predating on 5 small carps for 6 hours, success ratio and number of prey captured (mean ± SD, significantly different from control : * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

| | dominant individual | | | subordinate individual | | |
|---|---|---|---|---|---|---|
| exposure time (h) | 0 | 24 | 96 | 0 | 24 | 96 |
| resting (min) | 222.99 ± 22.74 | 228.24 ± 44.39 | 254.88 ± 32.84*** | 280.18 ± 21.11 | 277.92 ± 13.87 | 262.88 ± 17.86 |
| agonistic behaviour (min) | 73.26 ± 7.97 | 68.12 ± 7.24 | 52.73 ± 13.46*** | 0 | 0 | 0 |
| predation (sec) | 31.10 ± 7.37 | 31.50 ± 6.63 | 18.90 ± 2.23** | 8.00 ± 1.00 | 6.75 ± 0.51 | 0 |
| swimming (min) | 83.70 ± 28.14 | 79.73 ± 42.11 | 31.54 ± 32.29* | 27.03 ± 19.58 | 30.57 ± 16.78 | 23.92 ± 14.39 |
| prey handling (sec) | 7.44 ± 2.25 | 8.12 ± 2.55 | 10.91 ± 3.98* | 7.00 ± 1.00 | 7.75 ± 1.89 | 0 |
| success ratio | 0.96 ± 0.08 | 0.89 ± 0.11 | 0.86 ± 0.21 | 0.75 ± 0.50 | 0.80 ± 0.44 | --- |
| number prey captured | 4.30 ± 0.48 | 4.00 ± 0.47 | 1.90 ± 0.56*** | 0.30 ± 0.48 | 0.40 ± 0.51 | 0 |

Table 2: Plasma and white muscle ammonia level in brown trout after exposure to 1 mg $l^{-1}$ ammonia for 24 and 96 hours or control (0 h). * is significantly different from control (mean ± SD, $p<0.05$).

| | Ammonia content white muscle (µmol $g^{-1}$) | | | Ammonia content plasma (µmol $l^{-1}$) | | |
|---|---|---|---|---|---|---|
| exposure time (h) | 0 | 24 | 96 | 0 | 24 | 96 |
| small individuals | 1.22 ± 0.21 | 1.52 ± 0.24 | 1.74 ± 0,45* | 124.6 ± 32.57 | 386.49 ± 56.74 | 401.43 ± 62.58* |
| large individuals | 1.53 ± 0.33 | 1.97 ± 0.35* | 2.03 ± 0.53* | 153.54 ± 34 | 412.76 ± 45.42* | 487.76 ± 54.24* |

($U_{max}$ = 175.8 cm $s^{-1}$, $A_{max}$ = 34.1 m $s^{-2}$, $D_{tot}$ = 83.8 ms). The present study shows that ammonia exposure for 96 hours significantly reduces maximum velocities in fast starts in large and small fish. From an ecological point of view, it can be stated that a reduced fast start performance might have an effect on escape and predation. Walker et al. (2005) showed that faster fast starts increase the probability of evading predators. Thus, a reduced speed could lead to a higher risk to be captured by a predator. Consequently, predators, not affected by ammonia concentrations in the water, like avian predators or mammals, could have a significantly increased success in predation on brown trout in ammonia polluted freshwater.

Directionality of escapes and/or predation fast starts can be discussed with respect to three parameters: initial orientation of the escape response, i.e. 'towards' or 'away', average turning rate and final orientation. The results on directionality in small fish escape responses showed to be mainly 'away' from the direction of the stimulus (i.e. between 90 and 270°) in clean water and after 24 hours of exposure, but large fish already show 50% 'towards' responses after 24 hours of exposure. The final orientation of the escape response of unexposed trout of both size classes and small trout after 24 hours of exposure showed a significant average around 170° from the stimulus, while exposed large trout and small trout after an exposure of 96 hours showed a random distribution. This is also an indication of a disrupting effect of ammonia exposure on the success of escapes in trout.

Comparison of the data according to body size shows that maximum C-start velocity is related to body size. This is in accordance to Wardle (1975), who showed that burst speed is related to minimum muscle contraction time, being in turn related to fish size. Also, total distance of a fast start is size dependent because bigger fish perform longer fast starts (Webb, 1976).

*- Predation fast starts -*

Results on predation fast start variables show a decrease in $U_{max}$ and $A_{max}$ after 96 hours of exposure. Also, $D_{100}$ is significantly reduced but not $D_{tot.}$ This is due to the increased duration of predation fast starts after 96 hours of exposure but not the distance to the prey when the fast start was elicited. However, as $D_{100}$ is the variable that has more effect on possible predation success (Webb, 1976; Domenici & Blake, 1993), the results show a negative effect of ammonia exposure on predation performance. As performance of escape fast start is reduced with exposure to an increased ammonia concentration, this should affect predator prey relationships in nature. Walker et al. (2005) showed that faster escape fast starts increase the probability of evading a predator. Thus, predators have to strike fast, in order to be successful. The present study shows that exposure to ammonia reduces not only escape but also predation fast start performance. Therefore, it can be assumed that a reduction of predation fast start performance can lead to a lower success rate in nature, especially when fast start performance of the prey is not affected by ammonia concentration in the water. To our knowledge, there are not many studies on predation fast starts. The few studies published (e.g. Webb and Skadsen, 1980; Rand and Lauder, 1981; Harper and Blake, 1990; Harper and Blake, 1991) present similar values on feeding strikes as found in the present study. Even though these studies present feeding S-starts in pikes (Genus *Esox*), our results on trout show feeding strikes that are very similar to a feeding strike observed by Harper and Blake (1991, feeding fast start IV). However, as the feeding strike did not have much similarity to an S-start described by Webb (1976) in rainbow trout, it still can be generated by means of white muscle action without being innervated by Mauthner cells, seeing the high $U_{max}$ values reached. According to Domeici and Blake (1997) feeding fast starts are unlikely to be generated by Mathner cells because of their repetitive movement patterns.

The exposure to ammonia at high concentrations also could influence the hunger level and therefore the motivation to attack prey. This would explain the delayed predation action after exposure when presenting the trout with the bait. However, the starvation time of 96 hours should be sufficient to elicit a predation fast start with maximum performance, also indicated by maximum velocity and acceleration values found that are comparable to maximum values at escape fast starts in our study and in the literature (e.g. Webb, 1976).

*- Predator prey interaction -*

Studies about fast start and escape performance are generally one-sided, highlightening only the performance of the prey, and ignoring possible effects of experimental factors (e.g.: hypoxia, toxins, reduced visibility) on the predator (e.g. Lefrancois et al., 2005, Meager et al., 2006, Weber, 2006). Thus, it can be argued that there might be no change in encounter rate and mortality of the prey population as the performance of the predator is altered in the same way. The present study reveals that agonistic and predation behaviour of trout is reduced with increased ammonia concentration in the water. Quigley (1975) showed that an increased ammonia concentration reduced the number of agonistic acts between individuals of rainbow trout. Israeli-Weinstein and Kimmel (1998) reported that exposure to much higher ammonia concentrations than used in the present study altered behaviour in common carp. Therefore, the ammonia concentrations used in this study do not have any impairing effect on the swimming and escaping capacity of the prey, i.e. the carp. Moreover, observation and monitoring of behaviour of the predator reveals that increased ammonia concentration alters intra- and interspecific behaviour and thus reduces predation rate and mortality of the prey. Therefore, as brown trout is known to predate while migrating (Lucas and Baras, 2001), an impairing effect of increased ammonia concentrations on predator prey interactions can be analysed only specifically with respect to the predator prey couple.

As shown, trout is a sensitive fish and swimming capacity is reduced (Shingles et al., 2001; Wicks et al., 2002; McKenzie et al., 2003) at a low ammonia concentration that does not affect other species like carp in our study. Thus, as different species react differently to the same concentration of ammonia in the water, it can be assumed that also predators of trout might be affected differently (e.g. lampreys and pike) or not at all (e.g. birds, seals and sea lions).

*- Ecological relevance -*

The concentration of 1 mg $l^{-1}$ used in this study is the concentration is the Belgian Water Quality Criterion for surface waters. According to the Flemish Environmental Agency (Vlaamse Milieumaatschappij, www.vmm.be), the values found in Flemish water bodies are often exceed this limit. This, among other factors, might explain why the population numbers of trout in Flanders have decreased (Dumortier et al., 2005). Moreover, Walloon populations might also be affected, as they use Flemish waterways to migrate towards the open sea. This study clearly shows the negative ecological impact of increased ammonia values on freshwater trout populations and may partly explain decreased population numbers in nature.

As the surface to volume ratio is higher in small than in large animals, it can be expected that small fish are more susceptible than large fish. The results show that small fish are less susceptible for ammonia than large fish. Possibly, juvenile individuals still benefit from an ammonia detoxification mechanisms from their embryonic phase (Steele et al., 2001). Alternatively, a higher metabolism in smaller individuals leads to a higher $CO_2$ excretion that reduces the pH at the gills. The surrounding water becomes more acidic leading to a higher amount of $NH_4^+$ which makes it easier for $NH_3$ to leave the body via passive diffusion, and/or harder for environmental ammonia to enter the gills (Wilson et al., 1994).

It can be concluded that a high ammonia concentration in freshwater leads to reduced fast start performance in brown trout. This has an effect not only on escape but also on predation performance and might therefore alter predator prey interactions. Moreover, ammonia exposure alters the behaviour of the predator and thus may impair predator prey relationships on a population dynamical level. The concentrations used in this study represent concentrations found in nature and are thus ecologically relevant. These effects of ammonia on fast start performance should, therefore, be considered when establishing guidelines for threshold concentrations.

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***Chapter 8***

# *General Conclusions and Recommendations*

## *General Conclusions*

Fish migrate in order to optimise their fitness by using different types of habitats. Migrating fish often have to pass man made obstacles across their migration paths. In order to do so, swimming capacity and energetic use must be adequate. If fish are not able to pass those artificial obstructions populations can be disrupted by destabilization and this can lead to effects such as bottleneck. Finally, populations can get isolated and species can get extinct. Therefore and in order to give recommendations for fututer research and management it is important to study aspects of swimming performance, swimming modes and energetic use in migrating and non-migrating fish species.

As swimming performance and energetics are different in different fish species, many dia- and potamodromous fish species should be considered when evaluating possible effects of barriers. To evaluate the ability of several species to pass barriers and clear obstacles, swimming capacity and energetics of different types of migratory species were tested. The studied species were: brown trout *Salmo trutta fario* and European perch *Perca fluviatilis* (long distance migrating); roach *Rutilus rutilus* and common carp *Cyprinus carpio* (short to middle range distance migrating); gudgeon *Gobio gobio* (limited migration); bullhead *Cottus gobio* and stone loach *Barbatula barbatula*, (very limited to no migration). Critical ($U_{crit}$), optimal ($U_{opt}$) and maximum ($U_{max}$) swimming speed and oxygen consumption ($MO_2$) were evaluated in a Blazka-type swimming tunnel at 15°C and analysed, and the results showed values correlated to migration capacity with highest swimming capacity in trout and roach and lowest in stone loach and bullhead. The study shows that typical long term migratory species such as trout, perch and roach perform best in the swim performance tests. They show a relatively low SMR, a high scope for activity and high values for $U_{crit}$, $U_{opt}$ and $U_{max}$. Most likely, trout would have performed even better at a lower temperature, as indicated by the maximum swimming speed which was highest at 10°C. Roach

and perch performed excellent as well, especially the larger individuals. This is not surprising since they are typical long distance migratory species. Additionally, also gudgeon, considered to be a non-migrating species, perform surprisingly well. Gudgeon is a bottom dwelling species with limited migration behaviour, therefore we did not expect such high values. However, the scope for activity in this species was low. This suggests that a relatively large part of the $U_{crit}$ could have been fuelled by anaerobic metabolism. Therefore fast speeds might be less advantageous for this fish species in nature.

Aerobic metabolism and related swimming tests such as $U_{crit}$, and $U_{opt}$ gave no or very poor results in bullhead and stone loach, respectively, indicating that these species are no long term swimmers. It is shown that besides bullhead, which is already threatened in Flanders, stone loach is a species of concern when barriers, even small ones, are present. For technical reasons, critical swimming speed could not be measured for bullhead. When set into the swimming tunnel, this species set its pectoral fins in such a position that it could not be swept downstream by the water, an adaptation to its natural habitat, where bullhead lives between rocks and stones in fast streaming creeks and small rivers. It was shown that the main part of *Cottidae* muscles is glycolytical and this might be an explanation for the typical hopping movement they display. Bullhead also lacks a swimming bladder, another adaptation to its environment. Therefore, bullhead does not show any cruise swimming behaviour but always bursts for locomotion. Although bullhead is not generally considered to be a migrating species, there is evidence for migrations of several hundreds of meters by some active individuals. Like bullhead, stone loach also displaces itself by bursts rather than by continuous swimming. New data indicate that some individuals also perform migrations over several hundreds of meters. Both species seem to fit in the Behavioural Dichotomy Model which states that populations of fish have a stationary component of fish that remain in their home range, and a mobile component consisting of individuals without a fixed home range. These highly

mobile fish avoid genetic fragmentation of the population and are of vital importance for dispersal and recolonisation. Therefore, species as bullhead and stone loach are included in our study, and their swimming capacities should be taken into consideration when barriers are remediate by the use of fish passes.

Migrating fish species like the three-spined stickleback (*Gasterosteus aculeatus L.)* show distinct characteristics in their swimming capacities and energetics. Differences between migrating (Trachurus-form) and non-migrating (Leiurus-form) populations are significant and can be used to estimate the adaptation to migration within one species. Also, comparative studies of freshwater fish migration must consider seasonal and temporal factors which can make results difficult to interpret**.** The three-spined stickleback is a good model for studying migrating and non-migrating types from the same habitat at the same time. In contrast to North American sticklebacks where the migratory form displays enhanced long term swimming capacity ($U_{crit}$) and the non-migratory form enhanced short term swimming capacity ($U_{max}$), showing a continuum from benthic to limnetic individuals, the European three-spined stickleback of the trachurus type performs better in both, short and long term swimming with higher values of $U_{crit}$, $U_{opt}$ and $U_{max}$ in trachurus than in leiurus. This indicates a different adaptation of the European migratory form to its lifestyle, being a better long and short term swimmer and thus adapted to long diadromous migrations. European trachurus sticklebacks require the ability to escape predators but also rely on body armour to withstand predator attacks. They may not show the same flexibility as the non-migrating leiurus individuals for fast starts involved in escaping predators or predation. Despite the rigid body form of trachurus, this morphotype reached higher values of $U_{crit}$ and $U_{max}$ compared to leiurus. Migration to spawning grounds requires upstream swimming. It might be assumed that fish migrate at the speed with the lowest cost, at $U_{opt}$. In the present study, $U_{opt}$ differed significantly between the two morphs, with trachurus showing higher values than leiurus. This would indicate that trachurus can swim

at faster migration speeds at a relatively lower cost (*i.e.* energy consumed per distance swum).

Swimming at $U_{opt}$, trachurus types reached higher speeds than leiurus but consumed a greater amount of oxygen in absolute terms, which would seem unfavourable from a theoretical perspective. Yet the percentage increase in $MO_{2opt}$ compared to the total scope of activity between the two morphs was identical, indicating that the same percent of their aerobic energy budget was directed towards swimming at $U_{opt}$. Also, $CoT_{opt}$ was not significantly different, indicating that when swimming at the same speed, both morphs expended the same amount of energy, and that the elevated $MO_{2opt}$ value was only due to the increased SMR in trachurus. A possible reason for the higher energy demand at rest in migratory trachurus might be an increase in blood circulation carrying more nutrients and oxygen to the muscles, possibly due to faster heart rates.

Extrapolation to zero swimming speed showed higher values of SMR in trachurus compared to leiurus. By adjusting the SMR, fish could compensate for the energy demands of migration by allowing them, as is the case with trachurus, to reach a higher AMR and a higher scope for activity. The method of determining the SMR by extrapolating to zero velocity may bias estimates, and SMR could be overestimated if the swimming speed and $MO_2$ functions were elevated, or the regression slope was reduced due to inefficient swimming at low speeds. However, the two morphs of the same species were treated identically. Any error arising using this method should be the same for both morphs and thus ignorable.

Even though the fish were held under identical conditions and were fed sufficiently, trachurus showed higher liver lipid content compared to leiurus. It cannot be ruled out that the time of capture may play a role in the body composition differences found in this study. It therefore remains an open question whether trachurus has generally higher liver lipid reserves or just a seasonally higher physiological capacity to store lipids compared to leiurus. The

present results indicate that higher levels of lipids may be an adaptive trait of trachurus related to migration. Comparative studies of freshwater fish migration must consider seasonal and temporal factors making results difficult to interpret**.** The three-spined stickleback is a good model for studying migrating and non-migrating types from the same habitat at the same time.

When testing swimming capacity in the laboratory in order to estimate migration capabilities using an $U_{crit}$ test, the results should be interpreted with care. As shown, carp (*C. carpio* L.) can control its behaviour during the swimming test in a Brett-type swimming tunnel of a maximum length of three metres. The results show that the length of the swimming tunnel has an effect on the reached critical swimming speed because of an increased burst-and-glide period after gait transition in longer swimming tunnels. the nature of the increasing velocity test resulted in fish not only swimming at higher speeds but also to swim at these speeds for a longer period of time before being unable to swim off the downstream grid. Therefore, the combination of higher $U_{crit}$ and the longer time over which fish swam at burst-and-coast speeds, fish in the longer chamber would have performed much more work before becoming exhausted. This suggests that the effect of chamber length on performance is not primarily limited by physiological factors determining fatigue, but rather space constraints limiting the ability to execute the behaviour. Nevertheless, physiological factors undoubtedly also contribute to performance. Thus constraints in burst-and-coast swimming were unexpectedly affected by fish size. Short chambers were scaled to fish length while all fish swam in 3-m long chambers. As such, it might be expected that any spatial limitations on burst-and-coast surges would be greater for larger fish in the relatively for larger fish shorter 3-m long chamber. Instead, burst duration of larger fish was longer at a given swimming speed than for smaller fish in spite of the potential constraint on burst distance in the 3-m length chamber. Consequently larger fish covered a larger distance in a burst. Also, fish do not use the whole length of the swimming tunnel when burst-and-

gliding after gait transition, leading to smaller distances swum. Therefore, general size-dependent effects of chamber length will be factors adding to effects of speed increment and time interval on variation in $U_{crit}$ in the published literature. Similarly, if $U_{crit}$ is considered to be the speed which maximizes oxygen uptake, longer periods of bust-and-coast swimming probably using anaerobic metabolic pathways might underestimate $\dot{V}O_{2max}$ for fish swimming in longer chambers. As it is shown here, the gait transition was not affected by the different swimming chamber lengths. Therefore, such gait transitions could be a better reference for the comparison of metabolic performance among species.

Comparing BCF with MPF swimming fish, manoeuvrability, expressed as minimum turning radius ($R_T$) per swimming speed ($U$) is higher in angulliform swimmers than subcarangiform swimmers and the lowest in MPF swimmers. Results obtained from analysing digitised movie images of eel, sea bass and surfperch show that eels are the most flexible swimmers while surfperch are more rigid. When plotting $R_T$ against $U$ the data points show a random distribution but with a lower limit. The lower limit for $R_T$ is positively correlated with speed and shows that the sharpness of possible curves of swimming paths is decreasing with increasing swimming speed. The resulting slope of this lower limit is increasing with decreasing use of the trunk region for swimming. The anguilliform eel, using the largest amount of its trunk region for propulsion, has the lowest lower limit and thus can perform sharp curves even at high swimming speeds, while the MPF swimming surfperch, using its pectoral fins for propulsion and not the trunk musculature before gait transition is more limited in the sharpness of turns at higher swimming speeds. The present study shows that the rigidity of the body and the mobility and position of the control surfaces is particularly important for manoeuvrability. Also, in contrast to earlier founding, $R_T$ appears to be dependent on $U$. In conclusion it can be said that minimum turning radii are positively correlated with speed. As higher the body

flexibility and the use of the trunk for propulsion as lower is the minimum turning radius per speed.

Concerning swimming performance, this leads to the conclusion that at low speeds, BCF swimmers are more likely to escape predation and catch prey than MPF swimmers. However, migrating fish are generally assumed to migrate at intermediate to high speeds and therefore, the results of this study only allow an interpretation of speeds used for foraging and other behaviours involving slow locomotion.

The energetics of MPF swimming fish is very different from BCF swimming fish. In contrast to BCF swimmers, MPF swimmers correlate not speed, acceleration and deceleration, turning angle turning radius but pectoral fin beat frequency ($f_P$) with oxygen uptake when swimming at low to moderate speeds before gait transition. These are the results of a respirometry study correlated with digitised video imaging. The results suggest that energy requirements of a labriform swimming fish during spontaneous swimming can be accurately predicted ($r^2 = 0.71$) using the pectoral fin beat frequency. Also, future studies of metabolic rates and activity of free labriform swimming fish may benefit from including techniques that allow direct measurements of $f_P$ in the field. Given our results, we suggest that EMG records of the pectoral activity may be used to measure fin beat frequency (rather than speed and distance) and hence to estimate oxygen consumption in striped surf perch and other labriform fishes. The minimum metabolic rates found by extrapolating $f_P$ to zero activity resulted in values closely resembled in earlier studies of swimming energetics, using controlled swimming speed as a predictor. Thus, the similarity among the SMR estimates suggests a sufficient accuracy of the oxygen consumption measurements in the circular arena respirometer. Commonly, $\dot{M}O_2$ is measured in swimming respirometers at high swimming speeds. Work on forced linear swimming may be particularly relevant for pelagic fish that show long periods of relatively steady swimming in nature. However, it has been shown that the costs

of locomotion during spontaneous swimming in non-pelagic fish species are higher than that of forced swimming, due to the additional resistance components of the spontaneous swimming. Relatively low speed swimming as it occurs in nature may imply a high degree of manoeuvring, stability control and accelerations/decelerations with loss of momentum, particularly in fish that live in structurally complex environments. This may in particular apply to labriform swimmers, such as *Embiotocidae*, which rely on pectoral fin activity for all types of locomotion as well as a synchrony with their ventilation rates. As a result, the relationship between speed and $\dot{M}O_2$ during such activity patterns may be weak. This implies that the use of pectoral fins in striped surf perch in our experiment is mainly related to behaviours other than forward locomotion, including manoeuvring, stability control and hovering. In conclusion, pectoral fin beat frequency measured in the field by means of telemetric EMG can be used as a powerful tool for estimation of energy consumption in labriform swimming fish.
Finally, in fish, fast starts are used for escape and predation and are therefore an ecologically important movement. Fast starts are generated by glycolytical muscle performance and are influenced by many internal and external factors. It is known that ammonia pollution has a major effect on the glycolytical muscle action, thus creating conditions in which fast start performance might be reduced and predation rates altered. In C-starts, ammonia exposure has no effect on response latency. After 96 hours of exposure, cumulative distance, maximum swimming speed and turning radius were all significantly reduced and the direction of escape became at random. Predation strikes were also affected. Distance, speed and turning radius are significantly lower in exposed fish. Predator behaviour is also altered and the number of prey captured is reduced. The effect of ammonia exposure is more pronounced in large fish than in small fish.

This study shows that ammonia exposure affects brown trout escape response mainly through a reduction in fast start velocity and through an impairment of

directionality. Thus, in addition to a reduced strength of the response, ammonia exposure may also reduce the fish's elusiveness facing a predator. Predation rate and social interactions are disrupted and predator prey relationships may be altered.

When fish are migrating through a chemical barrier as a filed of ammonia polluted water, fast start capacity and anaerobic swimming capacity is reduced, leading to a lower migration speed and reduced energy uptake in form of foraging while migrating. It can be concluded that there are many factors, biotic and abiotic, which influence the capability of fish to clear man made physical or chemical obstacles across migration paths.

## *Recommendations for Future Work*

The present study highlights some aspects of swimming physiology and energetics involved in migration of several fish species. Based on the findings of the present work, future studies should involve the measuring of speeds and energy of migrating fish in the field. It is, for example, not known yet which the actual speed is that fish use for migration. Results of such a study could elicit vital information on migration behaviour and energetics in fish. Also, more relevant tests to measure ecologically important capacities of fish, such as swimming and migrating behaviour, measured under controlled circumstances, have to be taken into account. Critical swimming speed tests turned out to be too relative when comparing physiological and ecological factors on the swimming capacity of fish and therefore, new tests should be developed that give a more accurate comparison of swimming capacities. More toxicological research has to be done on environmental effects on migration as many migrating fish species show decreased population strengths due to reduced migration capabilities. Such studies are vital to investigate and protect migrating fish species and their habitat. Finally, only little is known about the physiology of migrating fish and evolutionary adaptations to changing environments. Relevant research could include changes in abiotic factors, such as salinity, pH and temperature, but also in biotic factors, such as species compositions of habitats and diseases. Especially two threats on endangered fish species should be included in this field of study, which could reveal vital information that can be used to better protect the environment: global warming and bio-invasion. As the environment is changing quickly, some migrating fish species do not have the possibility to adapt and therefore the effects of these changes on migration capacity have to be tested.

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