Tracking the behaviour and energy use of teleost fish: Insights from accelerometer loggers.

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Tracking the behaviour and energy use of teleost fish: Insights from accelerometer loggers

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This thesis is dedicated to Gail and Milton Wright

Abstract

Accelerometers are increasingly used in the study of fish behaviour and energy use. As tags become more accessible, the methods used to deploy tags and to define behavioural and energetic changes in fish must be validated to ensure that results are accurate and robust. The aim of this thesis is to explore these issues using a number of fish species as models. Firstly, the methods used to prepare tags, the behavioural response of fish to surgical implantation and issues associated with post-processing tag data are explored. The results show that (1) tags encased in medical-grade silicone reduce the incidence of infection and tag rejection, (2) the locomotor performance and stability of fish swimming improves with time from tag attachment, and (3) when splitting accelerometry data into static and dynamic motion, the optimal smoothing algorithm can be derived using automated procedures. The second section of this thesis quantifies the way in which accelerometers can be used to monitor energy use of teleost fish. Dynamic acceleration is an effective tool for measuring both aerobic and anaerobic energy use, providing a comprehensive method for remotely tracking the activity-specific energy use of fish. Additionally, accelerometers are used to monitor behavioural changes associated with environmental conditions and in relation to predation attempts, shedding light on the adaptability and flexibility of fish behaviour in response to biotic and abiotic changes. Findings from this research highlight the importance of methods to tag and process data in addition to showcasing some of the exciting methods which can be used to extract energetic and behavioural changes of fish, both in the laboratory and the wild.

Declaration

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed .... *(candidate)*

Date ..... Wednesday 13th May 2015 .....

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations

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Contents

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List of Figures

List of Tables

Chapter 1

Introduction

Serena R. Wright

Background

The factors that influence spatio-temporal movements of animals and the effect of both abiotic and biotic factors on these movements are crucial in our understanding of population dynamics (Beverton and Holt, 1993), seasonal abundance (Winemiller and Jepsen, 1998) and fitness (Payne et al., 2010). To monitor the behaviours and energy use of fish in the field, methods to track fish need to be robust, reliable and have a minimal effect on "natural" behaviours. This is complicated further because fish live in dynamic and often inaccessible environments making direct observations difficult, if not impossible.

Methods to track fish in the field have become increasingly affordable with advances in technology. In 1956, researchers from the National Marine Fisheries Service created one of the first tags solely aimed at tracking fish. The sonic transmitter enabled researchers to home-in on the fish location, resulting in a track of slightly over 1 hour over a distance of a few hundred yards (Trefethen, 1956). Since these early pioneering experiments, methods for tracking animals have improved both quantitatively and qualitatively, with a number of methods now in use, including animal-borne radio (Metcalfe et al., 2006), automated depth and temperature loggers (Hobson et al., 2007; Righton et al., 2010; Walker et al., 2000; Westerberg et al., 2013) and satellite-based positioning systems (Sulikowski et al., 2010). These new tracking techniques can be used to estimate locations worldwide with few temporal or spatial constraints (Cagnacci et al., 2010; Tomkiewicz et al., 2010).

For fish, which are particularly intractable species due to the nature of their environment, records of animal location and depth and temperature have been invaluable for identifying areas of importance based on residence times (Heupel et al., 2003) as well as for identifying migration routes (Hunter et al., 2004; Makiguchi et al., 2011; Metcalfe et al., 2006). However, the behavioural context of these movements is absent and must either be inferred from other recorded information or through direct observation. Thus, there is a need for tools to measure fine-scale behaviours of fish at a level of accuracy comparable to direct observation. To fill this knowledge gap, accelerometers are being increasingly used (Brownscombe et al.,

2013; Gleiss et al., 2011; Wilson et al., 2013), and are emerging as a reliable tool to study fish behaviour and activity both in the laboratory and the field.

Accelerometers register the acceleration and deceleration (change in velocity) of tagged animals, and thus provide an objective and direct measure of the intensity and frequency of movements during physical activity (Kawabe et al., 2003). However, as accelerometers are increasingly used in the study of fish behaviour and energy use (Fig.1), further research is required to explore the full potential of accelerometry and to validate methodologies. This thesis explores the way in which accelerometer tags can be used to gain insights into the behaviour and energy use of fish, with an aim to develop a robust toolkit which can be used to study fish in the laboratory and the field. There are three core themes to the thesis; (i) refining methods for deploying and analysing data from accelerometer tags, (ii) using accelerometers in the study of activity-specific energy use, and (iii) the study of fish behaviour. Firstly, methodologies for deploying accelerometers on fish are compared between species to identify best practise for tag attachment and the behavioural response of fish after tagging.

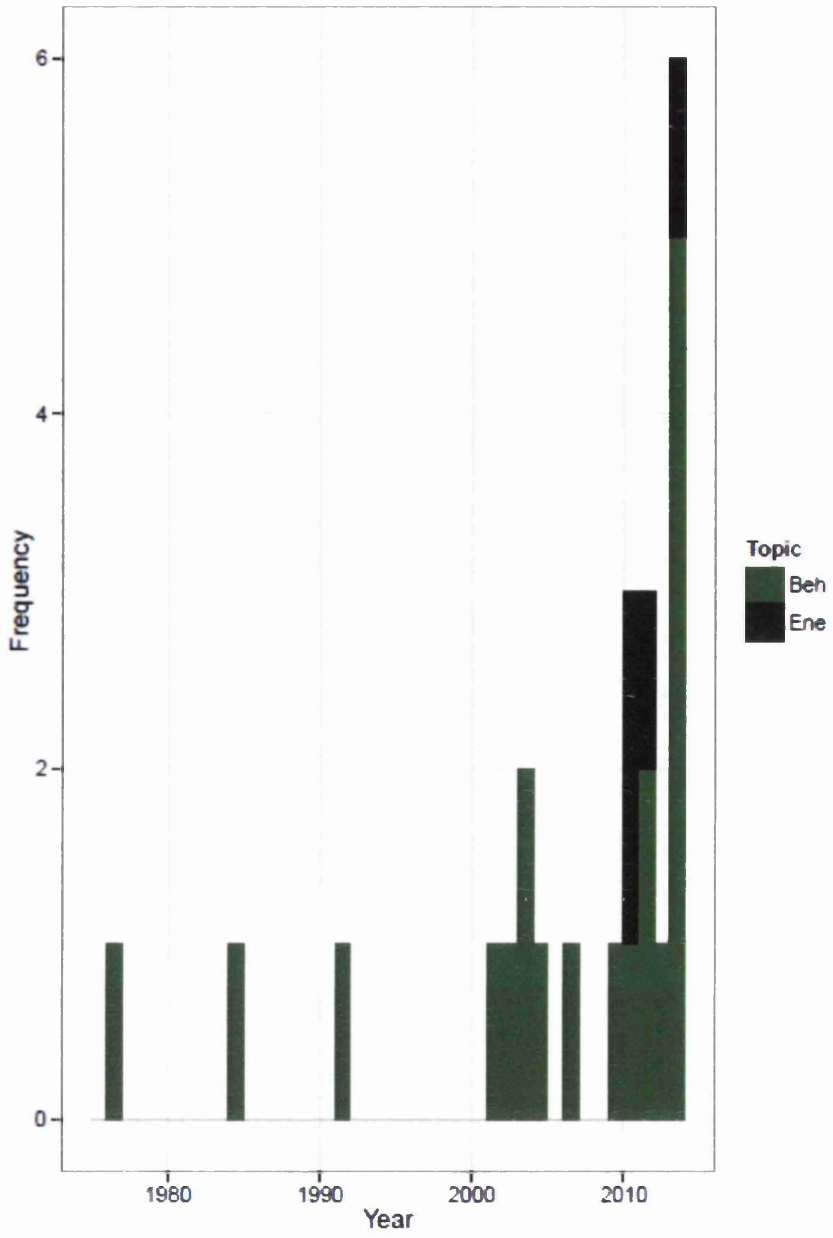

Figure 1.1. Number of publications by year featuring the use of accelerometers (uni, bi and tri-axial) for the study of fish behaviour (green) and energetics (purple).

I. Refining methods for deployment and analysis

In recent years, researchers from around the world have explored methods to attach and to analyse data from accelerometer tags (e.g. (Broell et al., 2013; Gleiss et al., 2009; Shepard et al., 2008a)). Both tag attachment methods and post-processing

analysis of acceleration need to be robust to avoid the misinterpretation of fish behaviour and physiology.

Accelerometers can be attached to terrestrial animals using a number of external tagging techniques including neck collars (Shepard et al., 2008b), leg bracelets (Passillé et al., 2010), harnesses (Halsey and White, 2010), tape (Brischoux et al., 2010) and glue (Brown et al., 2012). External attachment methods are also used for studies on fish and include the use of clamps (Gleiss et al., 2009), pins through the dorsal muscle (Broell et al., 2013) and nylon straps (Kawabe et al., 2003). These methods can be applied for short-term studies (the order of hours to days), though problems may arise due to bio-fouling (Dicken et al., 2011; Thorstad, 2001), an increase in drag (Thorstad, 2001) and the potential for tissue damage or abrasion (especially for relatively large tags) (McAllister et al., 1992). Thus, for long-term studies on animals, internal attachment likely represents a more practical method for tracking activity and behaviour.

Procedures for surgically implanting tags are increasingly studied and questioned (see Cooke et al., (2010) for review), though few have evaluated the effect of tag coating on recovery and tag retention for tags that must be fixed in place. By comparing the results of different coating techniques between individuals and species, I was able to identify the most effective method for tagging fish over long study durations to minimise the incidence of infection and tag rejection, whilst providing a stable and accurate measure of acceleration, as discussed in chapter 2. The behaviour of fish after tagging can also be derived from acceleration, ensuring that fish are provided with sufficient recovery time after tagging and before experimentation in the field or laboratory. Changes in activity level and swimming stability can be used to classify behaviours (Gleiss et al., 2013), and I used these as a tool to track behavioural change over time following surgery (chapter 2). I show that lack of provision for recovery time in fish tagging experiments can lead to misinterpreted behaviour and highlight how this sort of work is critical since assessment of how core behaviours change over time post tagging can indicate the value of long-term and complex behavioural and physiological studies using tags.

In addition to ensuring that tagging methods have a minimal effect on the behaviour and energy use of fish, post-processing methods also need to be robust to

ensure that behaviours are accurately defined. Tri-axial accelerometers record the acceleration of fish on three planes of motion corresponding to the surge (x), heave (y) and sway (z) of the fish (Fig.2). All three sensors collect simultaneous measurements, which are representative of three dimensional movement of the animal (Shepard et al., 2008b). This motion can then be used to distinguish between specific activities, with previous applications to humans (Foerster et al., 1999), cats (Watanabe et al., 2005), pigs (Cornou and Lundbye-Christensen, 2008), birds (Bom et al., 2014; Wilson et al., 2006) and fish (Broell et al., 2013). These behavioural classifications are based on two variables which are recorded by the accelerometer: (1) the dynamic acceleration and (2) the static acceleration. The static and dynamic acceleration reflect postural changes (Gleiss et al., 2011; Gleiss et al., 2013) and the short term changes induced from muscle contraction (Gleiss et al., 2010; Gómez Laich et al., 2011), respectively.

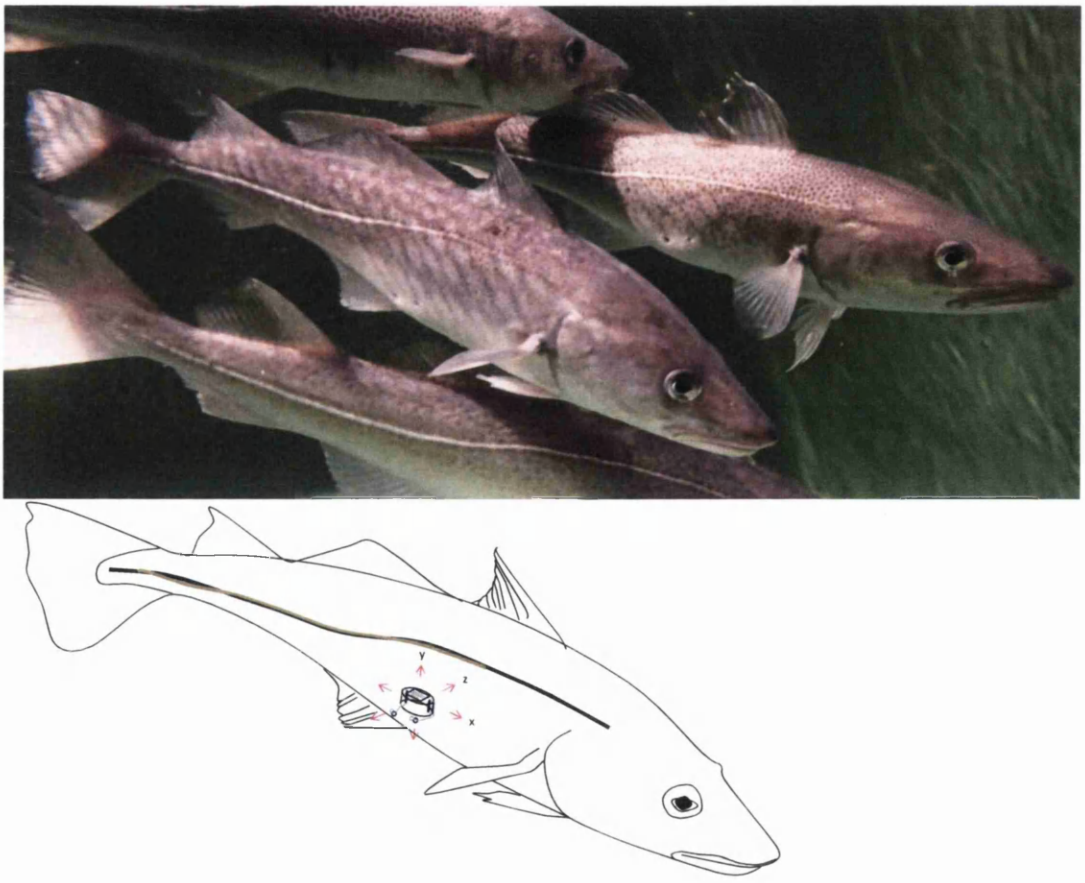

Figure 1.2. Tri-axial accelerometer in the body cavity of an Atlantic cod (*Gadus morhua*), showing the surge (x), heave (y) and sway (z) axes of motion.

To separate the static and dynamic acceleration, previous studies have used smoothing algorithms (Shepard et al., 2008a; Wilson et al., 2006) or pass-filters (Sato, 2003; Watanuki et al., 2003) although no studies to date have developed statistically robust methods for identifying the optimal smoothing algorithm or pass-filter limit. In chapter 3 I explore ways in which fish swimming simulations can be used to identify the most statistically robust method for extracting the optimal smoothing algorithm to separate static and dynamic acceleration in fish.

II. Acceleration and energy use

The energy budgets of teleost fish have long been of interest to behavioural ecologists (Godin and Rangeley, 1989; Hart, 1986), with energy use partitioned between a number of physiologically important costs including basal metabolic rate (BMR) (Frappell and Butler, 2004), specific dynamic action (SDA) (McCue, 2006), temperature regulation (Beamish and Trippel, 1990) and movement (Claireaux et al., 2006; Steinhausen et al., 2005; Svendsen et al., 2010). In recent years, the dynamic acceleration of animals has been shown to significantly correlate with oxygen consumption across a wide range of taxa including terrestrial mammals (Bouten et al., 1994), birds (Wilson et al., 2006), amphibians (Halsey and White, 2010) and most recently fish (Clark et al., 2010; Gleiss et al., 2010; Murchie et al., 2011; Wilson et al., 2013). Experiments linking the energy use of fish to acceleration began in 2010 with a total of five experiments having been conducted over the past four years (Fig.1; Clark et al., 2010; Gleiss et al., 2010; Murchie et al., 2011; Wilson et al., 2013; Wright et al., 2014). The interaction between dynamic body acceleration (DBA) and oxygen consumption varies depending on the species and conditions, however a significant correlation has been found for all studied species to date (Halsey et al., 2011). Thus, bodily acceleration derived from accelerometers can be used to assess qualitatively the amount of mechanical work performed by the body in different ecologically important scenarios.

The amount of mechanical work done must be balanced with the amount of energy coming in and the energetic cost of different behaviours. For example, to cover a given distance, fish can alter their propulsive motion to minimise energy use in response to the specific environment. This propulsive effort can be grouped into

three categories reflecting sustained, prolonged and burst locomotion (Brett, 1964). Thus, fish can cover a given distance by swimming steadily or by alternating between active swimming and gliding (burst-glide swimming) something that has proven especially important for fish that use tidal transport (Metcalfe et al., 2006).

The majority of studies which relate DBA with oxygen consumption are carried out during steady-sustained motion, reflecting the aerobic activity of fish (Wilson et al., 2013). Sustained swimming can be maintained for long periods (>200min) and is supported by well-perfused red musculature, using aerobic metabolic pathways (Brett, 1964). Burst swimming can also be used during locomotion and is almost exclusively reliant on anaerobic metabolism in the white muscle (Greek-Walker and Pull, 1975; Martinez et al., 2003). In contrast to steady motion, burst swimming can only be maintained for relatively short durations (<20 s) and can result in a significant reduction in intracellular energy supplies (oxygen, glycogen, fat etc.) and the accumulation of waste products including lactate (Hammer, 1995; Kieffer, 2000). Red muscle fibres have a high myoglobin content and a high content of mitochondria and oxidative enzymes (Bone et al., 1978) which enables the efficient generation of slow repetitive movements associated with low swimming speeds. White muscle has a low content of mitochondria and a poor blood supply, and thus white fibres fatigue rapidly (Blake, 1983). The relative importance of these muscle fibres has been studied for a number of species using velocity versus endurance curves (Hunter and Zweifel, 1971) with white fibres inactive at low speeds. Teleost fish vary morphologically, anatomically and kinematically and this is reflected in their ratio of white to red muscle (Greek-Walker and Pull, 1975). Active pelagic fish (i.e. scombriodae, clupeidae and carangidae) typically have a high proportion of red muscle (20 % of total muscle mass). In contrast, more sluggish species (i.e. anguillidae, and congridae) have a lower proportion of red muscle, such as Atlantic cod, *Gadus morhua*, which have just 1% of their total muscle mass comprised of red muscle (Videler, 1984). Differences in muscle composition and thus swimming capacity of teleost fish highlight the importance of quantifying energy use using both aerobic and anaerobic locomotion.

The applicability of accelerometers to track the aerobic energy use of fish has been assessed for a number of species including salmon (Clark et al., 2010; Wilson et al., 2013), bonefish (Murchie et al., 2011) and hammerhead sharks (Gleiss et al.,

2010), with a strong correlation between acceleration and oxygen consumption for all studies to date. However, further studies on new species are required to assess whether these relationships hold true across a number of anatomically distinct species and in association with changes in water temperature. In chapter 4, the correlation between DBA and oxygen consumption during aerobic locomotion is compared across a number of teleost species (sea bass, rainbow trout and European eel) and in response to temperature. In addition, I discuss scaling during aerobic energy use, the energy use during spontaneous activity, and how this affects species with a high proportion of white to red muscle.

Anaerobic energy use occurs as a result of high intensity behaviours including fast-starts (see Domenici, 2011 for review on fast-starts). These high force events can lead to higher than aerobically deliverable energy use, requiring increased post-exercise oxygen consumption, which was previously defined as oxygen debt (Hill et al., 1924). This involved an elevation in oxygen consumption in an attempt to repay the O_2 deficit which was ascribed to the oxidative removal of lactate. Oxygen debt is now defined more accurately as the excess post-exercise oxygen consumption (EPOC), and is classed as the elevated metabolism occurring after vigorous exercise (Gaesser and Brooks, 1984). The amount of EPOC and the time required to get back to pre-exercise levels has implications for the performance and thus survivability of wild fish. Few studies have identified how the intensity and duration of exercise affects the EPOC (Hancock and Gleeson, 2002), with fewer still assessing the amount of time required for oxygen consumption levels to return to aerobic limits (Lee et al., 2003). I have examined how accelerometers can be used to quantify the way in which the amount of energy exerted during exercise affects EPOC and recovery time (Chapter 5), with the concurrent effect of temperature.

III. Acceleration and behaviour

Movement is a fundamental behavioural response to both internal and external changes (Cooke et al., 2004). Technological advances are providing behavioural ecologists with new methods to track fish movement in the laboratory and field. Accelerometers, in particular, appear to provide a holistic means to monitor both overall and fine-scale changes in activity. Some of the first studies to

use accelerometers to track animal behaviour involved monitoring the biomechanics of motion in bluefish (DuBois et al., 1976), but, as accelerometers became less expensive and truly portable, behavioural tracking became more accessible with studies including quantifying behaviours of, for example, the running horse (Ratzlaff et al., 2005), flight in birds (Sato et al., 2009) and swimming in sea snakes (Brischoux et al., 2010), in addition to increasing the number of studies tracking the behaviour of fish using both acoustic (Baras et al., 2002; O'Toole et al., 2010; Taylor et al., 2013; Wilson et al., 2014) and logging accelerometers (Broell et al., 2013; Brownscombe et al., 2013; Gleiss et al., 2013; Noda et al., 2014; Tanoue et al., 2012), with a peak in studies in 2013 (Fig.1).

Accelerometers can be deployed in tandem with other sensors to obtain an environmental context to observed behaviours which can exceed the descriptive abilities of simple human observation. For example, accelerometer defined behaviours can be related to other factors including positional information (Wilson et al., 2013), and environmental context such as light-level and temperature, which can then be used to define environmental rhythms (Erkert and Kappeler, 2004).

Teleost fish are ectotherms and thus their energy budgets are intrinsically influenced by water temperature (Claireaux et al., 2000; Hein and Keirsted, 2012; Morgan et al., 2010). Therefore, we may predict that energetic costs such as locomotion will be affected by changes in environmental conditions like ambient water temperature. For example, Atlantic cod live in dynamic and thermally diverse habitats. Temperature changes as experienced by individual fish, occur not only seasonally but also abruptly, as fish travel across fronts (Righton et al., 2010). The effect of travelling between different water bodies and thermally distinct environments may affect locomotor activity (Claireaux and Lagarde, 1999). In addition to differences with temperature, it may also be anticipated that the behavioural responses would vary between geographically distinct stocks, with a preference for lower temperatures for cod in more northerly latitudes. To this end, I studied the locomotor activity of cod from the southern North Sea and the Northeast Arctic and compared this over a range of temperatures and in response to abrupt temperature changes in chapter 6.

Relative changes in activity level provide insights into the amount of energy that fish expend. Fast-starts can play a large role in these costs, which involve the contraction of anaerobic muscle fibres over short time frames (of the order of micro seconds). In relative time, fast-starts comprise a small proportion of behaviour, though they can play a vital role for many species where fast-starts are used for courting displays (Wong and Hopkins, 2007), predation attempts (Vinyard, 1982), escaping (Dill, 1974) and scaling otherwise impassable regions (for example salmon jumping up river). For example, great sculpin (*Myoxocephalus polyacanthocephalus*) use fast-starts during prey capture. As a means to compare the performance and outcome of predation attempts for great sculpin, I used both accelerometry and high speed video for comparisons both within and between individuals (chapter 7).

There remain many questions in terms of methodological practise, and the applicability of accelerometry in the study of energetics and behaviour. The work described in the following chapters attempts to address some of these questions by exploring methods to process acceleration data in addition to attempting to answer some previously unknown issues, including whether we can use accelerometers to track the anaerobic performance of fish. A number of teleost species were used in these analyses.

In summary, this thesis has been divided in 8 chapters. Starting with this chapter (chapter 1), chapter 2 then progresses by providing an insight into issues associated with tagging and the response of fish to surgical implantation of tags, while chapter 3 explores methods for processing acceleration to obtain accurate measures of activity level and postural change. Methods to estimate the energy use of teleost fish using steady-state aerobic exercise and high intensity anaerobic exercise are quantified in chapter 4 and 5, respectively. Chapter 6 quantifies the way in which accelerometers can be used to track the behaviour of fish in relation to environmental changes, chapter 7 tracks the behavioural changes associated with predation attempts for a sit-and-wait predator, the great sculpin, and finally chapter 8 concludes the study.

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Chapter 2

Internal tagging methods and locomotor activity with time from surgery

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S. R. Wright wrote the manuscript, collected and analysed the data. S. R. Wright, J. Metcalfe and J. Behrens conceived the study. J. Behrens and R. Wilson proof read the manuscript.

Abstract

Methods used to track animals need to have a minimal effect on the host. For internally implanted devices, the material that tags are made of can affect recovery and the likelihood of retention. The effect of tag coating was assessed in the laboratory for three species of fish, Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*). All fish were implanted intra-peritoneally using un-coated tags, or tags coated with medical grade mesh or medical grade silicone. Encapsulation occurred for all tags exceeding 28 days in duration, with a higher frequency of infection and expulsion for un-coated and mesh coated tags compared to tags encased in medical-grade silicone. Water temperature had a significant effect on the likelihood of tag expulsion with a higher risk of expulsion at higher temperatures. We suggest that medical-grade silicone coating is associated with higher retention times and reduced infections for tags implanted intraperitoneally in fish. Additionally, the behaviour of three Atlantic cod was tracked after surgery to assess whether accelerometers provided a means of tracking changes in locomotor activity with time from tagging. Cod were implanted with tri-axial accelerometers and were tracked for 8 days after surgical implantation. With time after surgery, fish became more active, though, notably the proportion of time spent at high activity levels (burst-swimming) did not increase with time.

Introduction

To obtain accurate measures of behaviour and energy use of fish in the field and laboratory, methods to observe and track animals need to have minimal effect. Fish tagging and tracking methods include external attachment (Broell et al., 2013; Gleiss et al., 2009), intragastric insertion using ingestion or forced feeding (Adams et al., 1998) and surgical implantation (Behrens et al., 2011; Thoreau and Baras, 1997; Wright et al., 2014). There is a general 2 % rule for tag attachment, whereby the tag should not exceed 2 % of the body weight of the fish in air, regardless of the attachment method (Winter, 1996). However, this 2 % rule may not be the only factor needing careful consideration, as other issues also affect tag suitability, including the planned experiment duration (Martinelli et al., 1998), environmental conditions, species (Butler et al., 2009) and amount of time available for tagging (Summerfelt and Smith, 1990), see Bridger and Booth, (2003) for review. For example, for juvenile salmonids, intragastric implantation is recommended for short-term studies and surgical implantation is considered more suitable for long-term studies, as gastric implantation has a chronic effect and surgical implantation has an acute effect (Martinelli et al., 1998).

For relatively long term studies, the surgical implantation of tags appear to have relatively minimal effects on survival, growth and behaviour on Atlantic salmon (Moore et al., 1990), Atlantic cod (Cote et al., 1999; Wroblewski, 1994) and pikeperch (Koed and Thorstad, 2001). Indeed, this work may explain why, in recent years, methods used for surgical implantation of loggers have received increasing focus (see Cooke et al., 2011 for a review), although relatively few have evaluated the effects of tag coating on recovery and retention (Cooke et al., 2011). A notable exception is a study involving rainbow trout (*Oncorhynchus mykiss*) where it was found that retention times were higher when tags were coated with bees wax than paraffin or silicone (Helm and Tyus, 1992).

Besides identifying appropriate tagging method and coating material for surgical implantations, it is also important to assess the behavioural response of fish to surgery and the amount of time required for fish to recover. Indeed, without appropriate recovery time, tag-derived behaviour and physiological changes can be misinterpreted, resulting in erroneous conclusions.

The aims of this study were firstly to identify the response and recovery of fish (bass, cod and trout) to surgical implantation of un-coated tags and tags coated in surgical mesh or silicone, and secondly to determine whether implanted accelerometers could be used as a tool to track the recovery of fish after tagging, specifically by examining behavioral changes in Atlantic cod (*Gadus morhua*) in semi-natural conditions. We hypothesized that as fish surgically implanted with tags healed after surgery, that they would suppress locomotor activity due to the increased energetic cost of tissue repair or as a response to pain or irritation after surgery (cf (Flecknell and Liles, 1991)).

Materials & Methods

I. Wound healing and tag retention

CTL G6a tags (40 mm × 28 mm × 16 mm, weighing 18.3 g in air, 4.3 g in sea water) were surgically implanted into European sea bass (*Dicentrarchus labrax,* n=28, mass range=1150 – 2650 g), Atlantic cod (*Gadus morhua*, n=32, mass range=1750 – 9010 g) and rainbow trout (*Oncorhynchus mykiss*, n=6, mass range=637 – 1066 g). G6a tags weighed 0.87±0.07 % (mean±S.E.) and did not exceed 2.87 % of the body weight of the fish (in air). Three methods were used to prepare the tags before insertion into the peritoneal cavity:

1. Un-coated, with the surface of the tag consisting of epoxy resin (Fig.2.1a)
2. Coated in surgical mesh (Polytetrafluoroethylene; Textile Development Associates, Inc., USA) (Fig.2.1b)
3. Coated in medical-grade silicone (734 Flowable Sealant; Dow Corning Corp.) (Fig.2.1c)

Figure 2.1. CTL (Cefas Technology Limited) G6a tags (a) uncoated, (b) coated in surgical mesh and (c) coated in medical-grade silicone.

Fish were anaesthetized with 2-phenoxyethanol using the method described in Wright et al., (2014). Tags were inserted through a 3 cm midline incision on the ventral surface of the fish. Independent of the method used to prepare the tag (described above as 1 to 3); tags were disinfected in 100 % ethanol followed by rinsing in a saline solution. Two sutures were connected to the right and left side of the tag and the sutures were guided to the flank of the fish using a biopsy needle, whereby the needle was passed along the outer edge of the body cavity before exiting through the muscle and skin on the lateral flank (Fig.2.2). Tags were positioned within the body cavity in alignment with the fish, as far from the ventral incision as possible. The sutures attached to the tag were tied off externally with two lock stitches. The incision was closed using interrupted sutures (coated Vicryl 2-0) and subsequently coated with antibiotic powder (Vetremox antibiotic powder with an Orahesive mix [50:50]). Fish were then transferred to a recovery tank where they were held in a stream of aerated sea/fresh water until they regained equilibrium. Sterile gloves were used at all times during surgery.

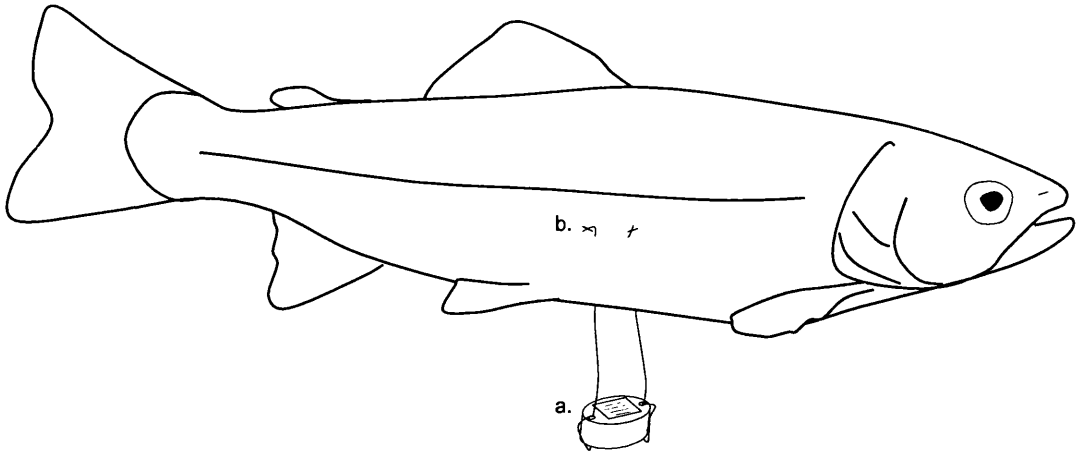

Figure 2.2. Schematic diagram showing the surgical implantation of a G6a accelerometer data tag. Two sutures are attached the to the tag (a.) and threaded through the body cavity along the peritoneal wall before being threaded through the left or right flank of the fish, where they are locked in place (b.).

Experiments with G6a tags were carried out between December 2010 and June 2013, with the duration of the studies varying from 6 to 207 days (Table 2.1). The outcome of each tagging experiment was sorted into one of three categories based on the severity of the reaction to the surgery:

1. Schedule 1 (fish survived until the end of the experiment)
2. Non-surgery related death (fish died from a non-surgery related cause)
3. Expulsion (tags were expulsed through the entry incision)

Previous studies have found an effect of water temperature on the likelihood of tag expulsion (Bunnell and Isely, 1999; Knights and Lasee, 1996), therefore temperature was also compared to the incidence of tag expulsion, in addition to the number of fish that showed infection, necrosis of the wound and tag encapsulation. With time from surgical implantation, tags may become encapsulated in connective tissue (Gheorghiu et al., 2010; Helm and Tyus, 1992), which may promote tag retention, internal tag migration or tag expulsion (Gheorghiu et al., 2010).

Table 2.1. Number of fish, duration and temperature of experiments involving sea bass, rainbow trout and Atlantic cod

Species	N	Duration (days)		Temperature (°C)	
		Minimum	Maximum	Minimum	Maximum
Bass	28	2	207	9.40	13.32
Trout	6	67	67	8.012	8.012
Cod	32	3	127	5.16	11.75

II. Recovery behaviour

Atlantic cod were used to assess whether there was a discernible change in behaviour and activity level in the 8 days after surgical implantation. In total three cod were used in this study, as the main aim was to assess whether accelerometers provide a tool to track the behavioral change of fish after surgical attachment of tags, rather than to define exact recovery times for the cod studied.

Animals

Adult Atlantic cod (n = 15, weight range 1360–4035 g) were caught by hook and line or gillnet in Skagerrak (10 °E; 58 °N) and transported in two 200L thermo-isolated tanks with continuously aerated 12-14 °C water to the North Sea Science Park, Technical University of Denmark, Hirtshals. Upon arrival, fish were kept in a 7 m^3 fiberglass tank supplied with flow-through fresh aerated seawater (12-14 °C) and a photoperiod of 8:16 h light-dark conditions. The fish were acclimatized for a week before tags were implanted. Post-operation, the fish were fed a mix of chopped fish fillet, squid and mussels every second day.

Surgery and accelerometers

Fish exhibiting normal swimming behavior, normal social interactions and no external injuries were chosen for experiments. Individual fish were anesthetized in water containing 0.133 g l^{-1} 3-aminobenzoicacid ethyl ester (MS-222) buffered with sodium bicarbonate (0.30 g l^{-1}) and transferred to an operating table covered with soft water-soaked rubber foam. The gills were continuously irrigated with aerated saltwater (8-10 °C) containing MS-222 (0.066 g l^{-1}) buffered with sodium bicarbonate (1.5 g l^{-1}). Fish were surgically implanted with G6a tri-axial accelerometer data tags (CTL Ltd.), see Wright et al., (2014) for tagging procedures. Tags were set to record acceleration at 30 Hz for 8 days after implantation, with the first 24 h unrecorded.

Experimental setup

Fish were left to recover for 24 h and thereafter transferred to an adjacent 92 m^3 aquarium with a photoperiod of 8:16 h light-dark conditions. The aquarium had two glass fronts enabling continuous video footage to be recorded throughout the

experimental period. In the midst of it was an artificial shipwreck overgrown by various sea anemones and algae, into and through which the fish could freely swim or hide. Besides the experimental animals, the aquarium housed Pollack (*Pollachius pollachius*), Saithe (*Pollachius Virens*), Ling (*Molva molva*), Atlantic wolffish (*Anarhichas lupus*), European plaice (*Pleuronectes platessa*), Lemon sole (*Microstomus kitt*), Atlantic halibut (*Hippoglossus hippoglossus*), goldsinny wrasse (*Ctenolabrus rupestris*), European lobster (*Homarus gammarus*) and European conger (*Conger conger*), around 35 specimens altogether. The fish were fed once a day at a specified time with a mix of chopped fish fillet, squid and mussels.

Data treatment & analysis

In order to estimate activity level, raw acceleration was processed to obtain dynamic body acceleration. The orientation of the tag was not aligned to gravity, so Vectorial Dynamic Body Acceleration (VeDBA) was used as the proxy for activity level rather than Overall Dynamic Body Acceleration (Qasem et al., 2012). Static acceleration was calculated using a 2 second smoothing algorithm through the raw acceleration on each of the three axes while the dynamic acceleration for each axis was derived by subtracting the static acceleration from the corresponding raw acceleration value in time. VeDBA was calculated as:

$$VeDBA = \sqrt{Dy_x{}^2 + Dy_y{}^2 + Dy_z{}^2} \quad (1)$$

where Dy_x, Dy_y and Dy_z correspond to the absolute dynamic acceleration of the fish on the surge, heave and sway axes of motion, respectively.

Depth was sampled at 1 minute intervals and was used to calculate vertical speed (VS), which was defined as vertical speed in metres min^{-1}. Tail beat frequency (TBF), was calculated as the number of tail beats per second and the stability and maneuverability of the fish was estimated by calculating roll:

$$Roll = \frac{180}{\pi} \cdot \arcsin(St_z) \quad (2)$$

where, St_z is the static component of acceleration of the sway axis, with the stability of the fish calculated as the standard deviation of the roll ($Roll_{SD}$).

The post-surgery locomotor activity was considered to be of particular relevance with regard to energetically costly activities like fast-starts (cf Domenici and Blake, (1997)). Therefore, the proportion of time at high activity levels (the top 20% of VeDBA values) was compared with time from surgery to assess the amount of time fish spent at relatively high activity levels.

Morphometric differences between individuals including total length (TL), weight (W) and Fulton's K condition factor were compared to assess whether differences between individuals were correlated to post-surgery behaviour. Condition factor (K) was calculated as;

$$K_i = \left(\frac{w_i}{l_i^3}\right) \cdot 100 \quad (3)$$

where, w_i is the somatic weight (g) and l_i is the total length (cm) of cod i. K is indicative of the nutritional state of the fish (Lambert and Dutil, 1997).

Overall changes in activity level (mean vectorial dynamic body acceleration, $VeDBA_{Mean}$ or mean vertical speed, VS_{Mean}) and swimming stability (Roll or $Roll_{SD}$) were compared in relation to time after surgery using general additive mixed effect models (GAMMs) on 5 minute averages of each variable. GAMMs were implemented using the mgcv library (Wood, 2006) in R (R Development Core Team, 2013). Individual cod were included in the model as a random effect to avoid pseudo-replication. A correlation structure was also incorporated to deal with the serial correlation of the time-series (autocorrelation), using an auto-regressive process of order 1 (AR1) (Zuur et al., 2010). The correlation at lag = 1 was used to specify the correlation structure of the GAMM. All behaviours (VeDBA and roll) were modelled as a function of time after tag attachment. The significance of the differences between individual fish was identified using a two-tailed t-test.

Results

I. Wound healing and tag retention

Un-coated tags were retained for up to a maximum of 2 months, mesh-coated tags for 7 months and silicone-coated for 4 months. Encapsulation occurred in all trials exceeding 28 days in duration, whereby tags became covered in a layer of tissue within the peritoneal cavity. Only 20 % of un-coated and 78 % of mesh-coated tags became encapsulated compared to 100 % of silicone-coated (supplementary material Fig.S2.1).

There was a higher incidence of tag expulsion for un-coated (70 %) and mesh-coated (17 %) tags, whilst, all silicone-coated tags were retained (Fig.2.3). In total, 5 % of trials which ended in expulsion had encapsulation (supplementary material Fig.S2.2), with expulsions only occurring at temperatures exceeding 12.9 °C.

Overall, there was an increased likelihood that fish tagged with un-coated tags would develop an infection (10 %) compared to coated tags (4 %).

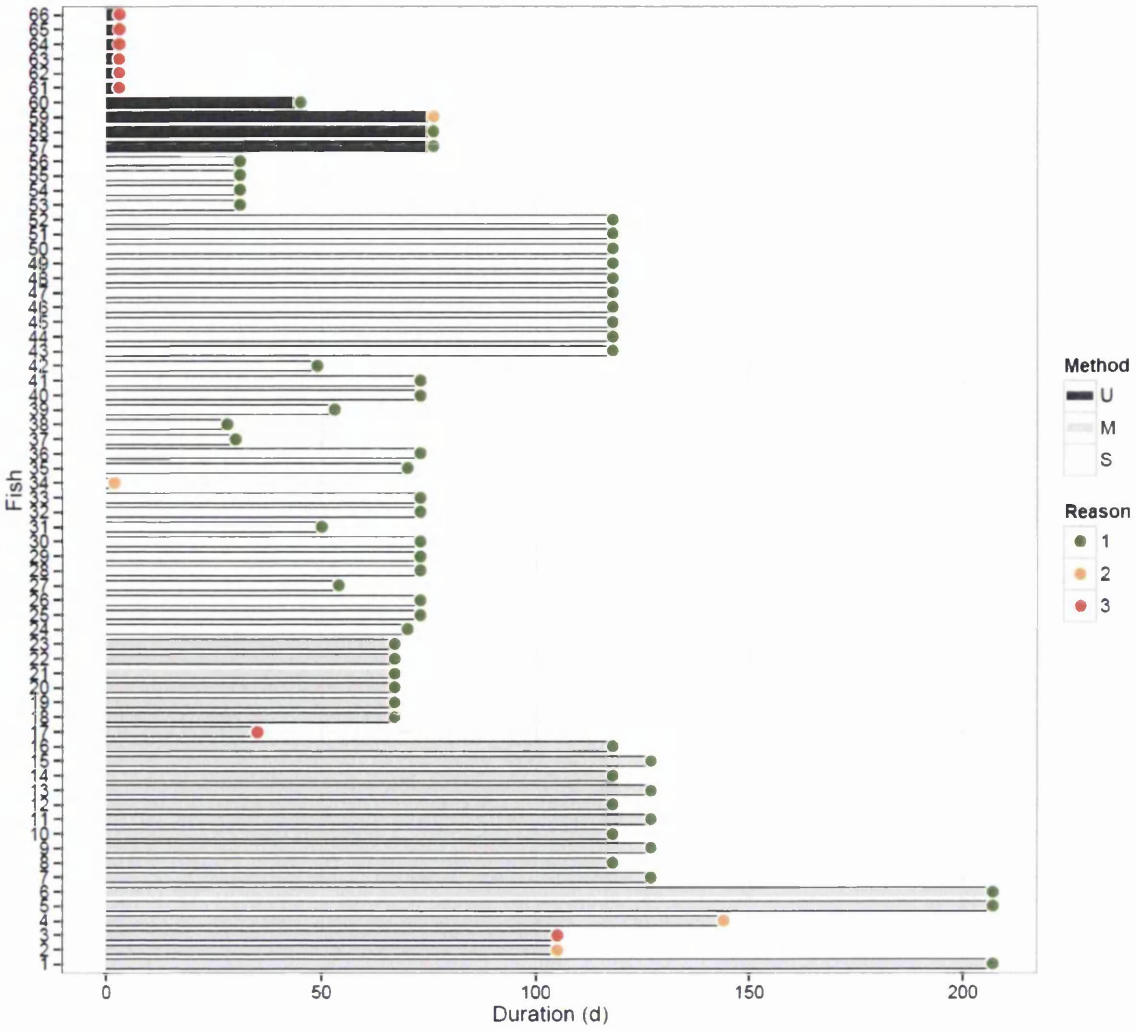

Figure. 2.3. Duration of experiment in relation to the coating method (U=Un-coate, M= Mesh-coated and S=Silicone-coated) and the severity of the reaction to the surgery (1= Survived until the end, 2= Non-surgery related and 3 = Tag expulsion).

II. Behaviour after surgery

After 8 days, only one cod had food in its stomach (Table 2.2). With time from surgery, there was a significant change for all variables except for mean roll (Table 2.3).

Table 2.2. Individual cod characteristics and the proportion of time at high activity levels during recovery.

Fish ID	TL (cm)	W (g)	Ventricle Mass (g)	Condition factor (K)	Internal Parasite	External Parasite	Stomach contents	Time at high activity (%)
1	58.0	1930	1.34	0.989	0	0	Empty	0.159
2	70.0	3560	2.74	1.038	0‡	1	Empty	0.275
3	61.0	1940	2.11	0.855	0†	0	Full	0.627

‡Scar tissue in atrium

†Heart infection (white dots) and gill damage

Activity level ($VeDBA_{Mean}$ and VS_{Mean}) and swimming stability ($Roll_{SD}$) were lowest immediately after surgery and became higher with time after surgery (Fig.2.4), although there was a reduction in activity and swimming stability on day 5. The disassociation between tail beat frequency and $VeDBA_{Mean}$ suggests that the increase in $VeDBA_{Mean}$ as fish recovered was a result of an increase in tail beat amplitude rather than tail beat frequency.

Table 2.3. General Additive Mixed effect Model (GAMM) using Gaussian error structures and a sampling window of 5 minutes

Variable	Resample function	Correlation at lag 1	Degrees of freedom	*F*-statistic	% deviance explained	*P* value of smoother
$VeDBA_{Mean}$	Mean	0.759	7.108	14.34	7.76	< 0.001
TBF_{Mean}	Mean	0.711	8.098	12.92	7.82	< 0.001
$Roll_{Mean}$	Mean	0.866	1.000	0.10	0.00	0.752
$Roll_{SD}$	Mean	0.788	4.897	37.81	9.38	< 0.001
VS_{Mean}	Mean	0.590	7.444	36.24	11.90	< 0.001

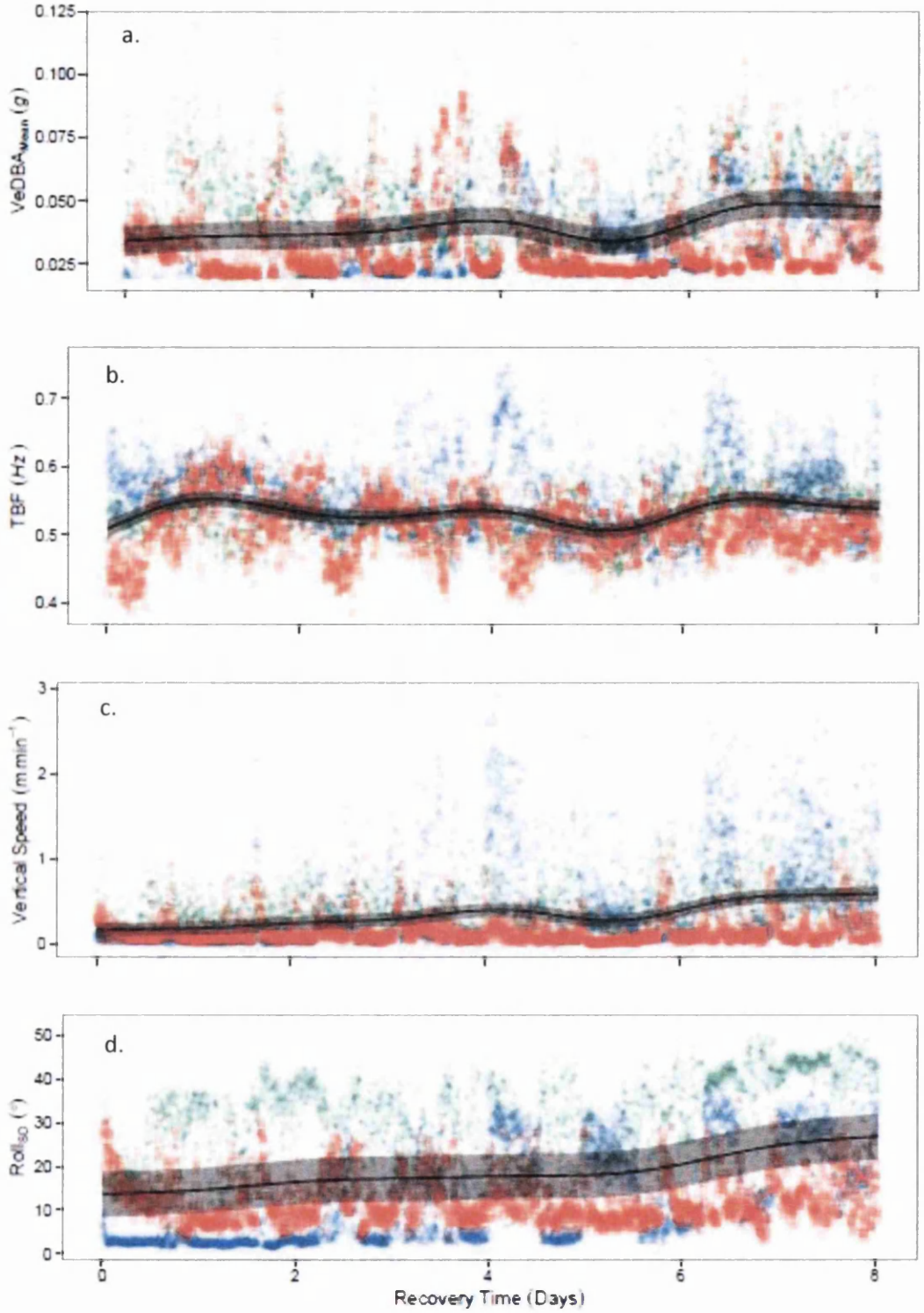

Figure 2.4. General additive mixed model of 5 minute means for vectorial dynamic body acceleration (VeDBA) (a), tail beat frequency (TBF) (b), vertical speed (c) and standard deviation of the roll ($Roll_{SD}$) (d) for three Atlantic cod (*Gadus morhua*). VeDBA. Vertical speed and $Roll_{SD}$ were lowest at the start of the study and highest at the end. TBF was also low at the beginning of the study, but varied substantially throughout the trial. Symbols represent cod 1 (green), cod 2 (blue) and cod 3 (red).

The proportion of time that the locomotor activity was in the upper 20 % (high activity) was compared between individuals after surgery. There was a significant difference between the three fish, with the highest activity for fish 3 with a full stomach (0.6 %) and lowest condition factor (Fig. 2.5, Table 2.2). Fish 2 showed a declining trend in the proportion of time spent at high activity levels with time from surgery (Fig. 2.5).

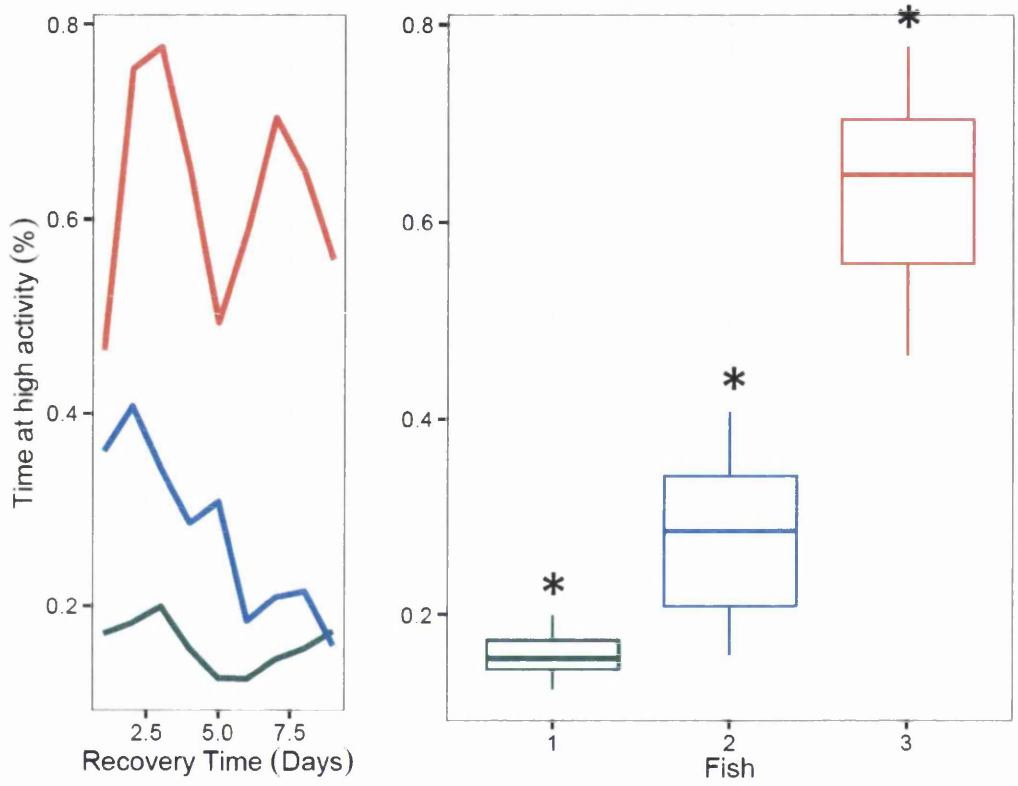

Figure 2.5. Proportion of activity in the upper 20 % as cod recover from surgical implantation. Individuals are shown as different colours, with significant differences between individuals are denoted by asterisk above the boxplot.

Discussion

I. Wound healing and tag retention

The results of this study highlight the importance of using appropriate coating for internally implanted tags which are fixed within the body cavity. Long-term studies with accelerometer tags require that tags remain fixed in place to maintain a stable reading of acceleration and thus an accurate measure of acceleration-derived metrics. To anchor the tag within the body cavity, two sutures

can be attached during surgery, though additional encapsulation during recovery may aid in stabilisation. Encapsulation is a characteristic reaction to a foreign body and has been shown to occur prior to expulsion (Coleman et al. 1974). Tags became encapsulated in all trials exceeding 28 days in duration. Previous studies also note encapsulation after tag implantation for a number of other species including catfish (Summerfelt and Mosier, 1984), rainbow trout (Lucas, 1989), Atlantic salmon (Moore et al., 1990), tilapia (Thoreau and Baras, 1997) and Atlantic cod (Cote et al., 1999); however, 95 % of tag encapsulations did not result in expulsion for fish in this study.

The 5% of trials that resulted in expulsion occurred through the incision, rather than transintestinally, through the body wall or anus (Baras and Westerloppe, 1999; Moore et al., 1990). Expulsion through the incision rather than any other site may relate to the relatively large size of the tag, as the incision may have been the only possible site through which the fish were able to expel the tag. When retention was compared between coating methods, silicone was the only coating agent which did not result in expulsion. Previous studies have also shown that the material used to coat the tags affects tag retention (Helm and Tyus, 1992), with bees wax coated tags more likely to be retained than paraffin- or silicone-coated for rainbow trout, *Oncorhynchus mykiss* (Helm and Tyus, 1992). We did not test the applicability of bees wax as a coating method, although there was a high retention rate observed when rainbow trout were tagged with silicone-coated tags, which suggests that medical-grade silicone can be used as an effective coating material for this species.

Water temperature had a significant effect on the likelihood of tag expulsion with expulsion only apparent when temperatures exceeded 12.9 °C. Previous studies also note increased risk of tag rejection with increases in temperature for rainbow trout (Bunnell and Isely, 1999), bluegills (Knights and Lasee, 1996) and striped bass (Walsh et al., 2000). Thus, although, higher temperatures facilitate wound healing (Andersens & Roberts 1975), there may also be an increased risk of tag loss (Knights and Lasee, 1996).

II. Recovery from tagging

Cod behaviour was compared over time following surgical implantation using accelerometer tags. Cod were housed together in a large aquarium facility at Hirtshals, Denmark, providing semi-natural conditions for the study. Locomotor activity was suppressed after surgical implantation, as has been noted for tilapia, *Oreochromis aureus* (Thoreau and Baras, 1997), though other energetically costly events including air exposure also result in a similar reduction in activity (Arlinghaus et al., 2009). Decreases in activity after surgery or air exposure may be attributed to increases in physiological costs resulting from metabolic, acid-base and ionic changes. Thus, with time from surgery, the energy reserves available for other physiological or behavioural functions such as locomotion may increase (Martinelli et al., 1998) causing a concurrent increase in activity level.

Even with increases in mean activity level, the proportion of time spent at high activity levels did not increase with time from surgery. Though, the fish with the full stomach and the lowest condition factor spent the highest proportion of time at high activity level, potentially reflecting an increase in the use of fast-starts during feeding events.

The standard deviation of the roll was used as a measure of fish stability, however the link with fish activity level (both VeDBA and vertical speed) suggests that the $Roll_{SD}$ and fish activity are not exclusive. For example, more active fish will use a larger range of roll angles which will appear as less stable locomotion, or increased maneuverability (Fish, 2002).

Conclusion

Results from this study suggest that internally implanted tags should be coated with biologically inert material, such as medical grade silicone, to reduce the risk of infection and to ensure that tags are retained for as long as possible, especially at higher temperatures. Tags coated in medical-grade silicone were most likely to result in both encapsulation and retention for sea bass, rainbow trout and Atlantic cod.

Additionally, acceleration is shown to be a useful tool for identifying behavioural changes of fish in response to important events, such as recovery from surgery. Previous studies have tracked behavioural changes of fish during recovery

using video analysis (Kavitha and Venkateswara Rao, 2007). In contrast to video analysis, accelerometers provide a means to remotely monitor the orientation and activity level of fish. As tags become smaller and attachment methods become less intrusive, it should be possible to track behaviours of fish in response to important events including the capture and release of fish from both recreational and industrial fisheries, providing information about behavioural state including whether fish are active or stationary and their stability during swimming. For example, a recent study by Brownscombe et al., (2013) has shown how accelerometers can be used as a tool to track the locomotor activity and behaviour of bonefish, *Albula spp.* in response to the methods used to retain and allow fish to recover before release. Future studies should assess the behaviour over a longer recovery period with more individuals to obtain a more accurate overview of behavioural responses to tagging.

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Chapter 3

Separating dynamic and static acceleration for steadily swimming fish: Insights from simulated data

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S.R. Wright was responsible for data collection, analysis and writing the manuscript. Additional data was collected by N. Whitney, J. Behrens, E. Sandblom, A. Gräns, M. Axelsson, C. Tudorache and A. Gleiss. All authors proof read and made amendments to the manuscript.

Abstract

Acceleration is increasingly used to study fish behaviour and physiology, with the potential to estimate both postural states and activity-specific energy expenditure of fish in the wild. To accurately separate these two components, we need robust methods to partition the raw acceleration into static (gravity-dependent) and dynamic (fish acceleration-dependent) acceleration. This study used simulations of typical acceleration patterns of fish using carangiform/ subcarangiform/ anguilliform modes of propulsion, to identify a method for deriving the optimal smoothing algorithm for steadily swimming fish. The method was then applied to swaying acceleration data from seven species of fish ranging in length from 0.38 to 8 m. Optimal smoothing windows for these fish ranged from 1.71 seconds (rainbow trout, *Oncorhynchus mykiss*) to 6.35 seconds (whale shark, *Rhincodon typus*), with a positive scaling relationship with fish length and a negative relationship with tail beat frequency. We provide a reproducible method for identifying the optimal smoothing algorithm for fish swimming at different speeds, with the aim to improve the methods used to estimate the behaviour and energy use of fish both in the laboratory and the field.

Introduction

The energy budgets of free-ranging ectotherms such as fish, are affected by changes in both biotic and abiotic variables. To quantify such dynamic energy budgets we need tools to estimate metabolic rate and behaviour of fish both in the laboratory and in the field. Electromyogram (EMG) telemetry (Cooke et al., 2004; Hinch et al., 1998) as well as heart rate (f_H) (Clark et al., 2010) and acceleration logging techniques (Gleiss et al., 2010; Wilson et al., 2013) have previously been used to estimate metabolic rate, with pros and cons for each method depending on the study aim and system. Accelerometers can be used to estimate both activity-specific metabolic rate (Gleiss et al., 2010; Wilson et al., 2013; Wright et al., 2014) and fine-scale behavioural changes (Broell et al., 2013; Gleiss et al., 2011b). However, as acceleration is increasingly used in the study of fish ecology, the analytical procedures to process this data must be refined and adjusted in accordance with new findings.

Raw acceleration data is mainly influenced by static (derived from gravity) and dynamic (derived from the movement of the tag) acceleration. The dynamic component provides a metric for activity level, which can be related to movement-induced energy expenditure for both terrestrial (Gómez Laich et al., 2011; Halsey and White, 2010; Wilson et al., 2006) and aquatic species (Clark et al., 2010; Gleiss et al., 2010; Green et al., 2009; Wright et al., 2014). The strong link between dynamic acceleration and activity-specific energy use reflects the contribution of muscle contraction to this dynamic component (Gleiss et al., 2011a). Static acceleration can be used to identify fine-scale postural changes (Gleiss et al., 2011b; Gleiss et al., 2013; Sato, 2003) since it corresponds to the orientation of the tag with respect to the earth's gravitational field.

Nevertheless, accelerometers measure both static- and motion-based acceleration and it is not trivial to disentangle these two elements (Shepard et al., 2008) not least because static and dynamic acceleration can co-vary. This is, however, essential as incorrect partitioning can lead to misinterpreted activity of tagged fish (Robson and Mansfield, 2014). Consequently we need robust methods to separate the (higher frequency) dynamic from the (lower frequency) static acceleration. In order to obtain an accurate measure of the static acceleration,

oscillations in dynamic acceleration corresponding to the predominant locomotion method (tail beat frequency in fish) must be removed. This requires that acceleration values be averaged over a period greater than one stroke cycle (i.e. a single tail-beat). Notably, however, the duration of a stroke cycle can vary within and between species both in response to biotic and abiotic factors in their environment and through ontogeny, as larger organisms tend to have a lower tail (or limb) beat frequency (Sato et al., 2007; Shepard et al., 2008).

Previous attempts to separate the dynamic from static acceleration have used high/low pass-filters (Sato, 2003; Watanuki et al., 2003), which attenuate frequencies below/above a defined cut-off limit and remove the static or dynamic acceleration depending on whether a low or high pass-filter is used. For these studies, the pass-filter limits are adapted to reflect the frequency of the dominant behavioural signal.

As an alternative to low/high pass-filters, smoothing algorithms can also be used (Shepard et al., 2008; Wilson et al., 2006), whereby the length of the smoothing window is adapted for each species and mode of locomotion. Smoothing algorithms are analogous to pass-filters; so a pass-filter with relatively high band limit is analogous to a running mean over a small number of data points.

To validate the methods used to process acceleration it is possible to compare estimates of postural change with high speed video recordings (Heath et al., 2006). However, this method is impractical for free-ranging species like fish, necessitating the use of other techniques. Another method that has been used extensively in selecting the optimal smoothing window is based on a two-tailed *t*-test to identify the point of stabilisation of mean dynamic or static acceleration as the smoothing window length is increased/decreased (Shepard et al., 2008). The two-tailed *t*-test relies on there being a sufficient (and significant) difference between adjacent dynamic or static acceleration values as the smoothing window increases (prior to the optimal smoothing level). For example, mean dynamic acceleration increases before stabilising around the optimum, resulting in significant differences between adjacent mean dynamic acceleration values before stabilisation. However, if the smoothing window length is increased in small steps, there may be no significant difference between adjacent mean dynamic acceleration levels prior to the point of stabilisation. The point of stabilisation may therefore be an artefact of the step-wise

difference between smoothing terms. Shepard and co-workers (2008) also noted that this approach was unlikely to resolve changes in body posture at a fine scale, but was useful for estimating dynamic body acceleration.

In an attempt to overcome the above described difficulties, the aim of this study was to develop a statistically robust method for selecting the optimal smoothing window to separate static and dynamic acceleration. To our knowledge, the present study is the first to use fish swimming simulations to validate methods for post-processing accelerometer data. Though it is not possible to establish whether the components have been attributed accurately to static and dynamic acceleration the study aims to standardise the approach taken by researchers. The method that produced smoothing terms closest to simulated static acceleration was then used on datasets from animal-attached loggers, providing scaling relationships between optima and animal size both inter- and intra-specifically.

Materials and Methods

Animals and data collection

Seven fish species were used to identify whether there was a scaling relationship between optimal smoothing frequency and fish size or tail beat frequency. The selected species were chosen as a representative group of fish swimming using carangiform, subcarangiform or anguilliform modes of propulsion and can be grouped into temperate and tropical species.

Teleost

European eel (*Anguilla anguilla*) were caught in the river Avon, Hampshire in June 2012. Eels were transported to Cefas, Lowestoft in an aerated tank with freshwater. At Cefas, fish were held in a 2 m^2 tank of aerated freshwater at ambient temperatures. After two weeks of recovery from the capture and transport, fish were equipped with G6a accelerometers. Tags were externally sutured in four places just behind the pectoral fins (Fig.3.1i) and were set to record tri-axial acceleration at 30 Hz for 24 hours.

European sea bass (*Dicentrarchus labrax*) were obtained from the Sea Life Centre at Great Yarmouth, UK, and held at the Cefas Laboratory, Lowestoft in a tank supplied with aerated seawater at ambient temperatures. Fish were equipped with G6a tags (Cefas Technology Limited Ltd., Lowestoft, UK) in February 2012. Attachment methods and tagging procedures are described in (Wright et al., 2014). Briefly, fish were fasted for 24 hours prior to tag attachment, at which time tags were implanted into the peritoneal cavity (Fig.3.1ii) where they were secured in place using two sutures. Tag attachment took no longer than 17 minutes and fish were given one month to recover before acceleration was recorded at 30 Hz continuously for two weeks.

Atlantic cod (*Gadus morhua*) were caught by hook or gillnet in Skagerrak in October 2012 and were transported in two 200L thermo-isolated tanks with continuously aerated 12-14 °C water to the North Sea Science Park, Technical University of Denmark. Upon arrival, fish were kept in 7 m^3 fibreglass tanks supplied with flow-through fresh aerated seawater (12-14 °C) with a photoperiod of 8:16 h light-dark conditions. The fish were acclimated to lab conditions for a week before experiments were initiated and fed a mix of chopped fish fillet, squid and mussels. Four of the healthiest fish were selected from the fifteen and G6a tags were implanted into their peritoneal cavity (Fig.3.1ii) using the same methods as described for European sea bass (Wright et al., 2014). Tags were set to record at 20 Hz intermittently for two weeks.

Rainbow trout (*Oncorhynchus mykiss*) were obtained from an aquaculture facility on 14th December 2012 and were reared at Cefas, Lowestoft, UK for six months prior to the start of trials. Tags were implanted into the peritoneal cavity (Fig.3.1ii) using the same method as described for the European sea bass (Wright et al., 2014). Tags were set to record at 30 Hz for the duration of the study (9 weeks).

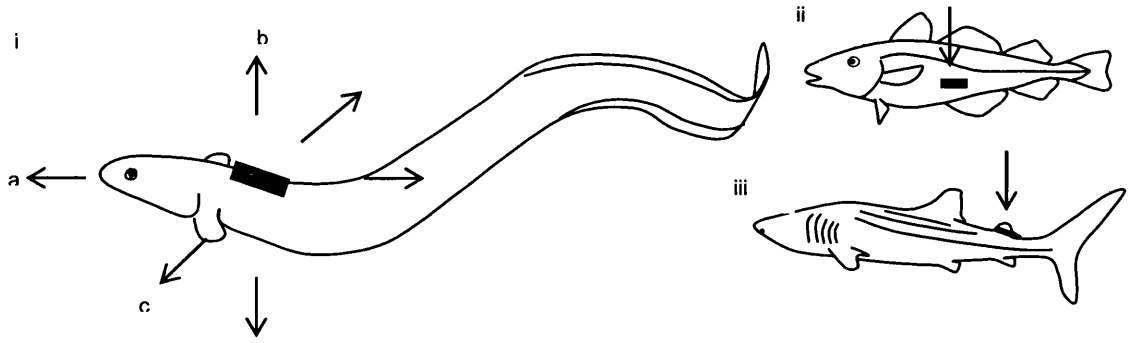

Figure 3.1. Schematic diagram showing the position of accelerometer data loggers (black rectangles) (i) behind the pectoral fins, (ii) within the peritoneal cavity and (iii) on the second dorsal fin for three different species of fish. The tri-axial accelerometers recorded acceleration on the surge (a), heave (b) and sway (c) axes.

Elasmobranch

A Blacktip blacktip shark (*Carcharhinus limbatus*) and two nurse sharks (*Ginglymostoma cirratum*) was were caught on drum lines offshore of Sarasota, FL, USA on 23rd in October 2009 and June 2010 respectively. All animals were maintained in a 151,000 l holding tank for experiments. Acceleration data loggers (Vemco, Nova Scotia, Canada) were attached to the second dorsal fin (Fig.1iii) using the method described in Whitney et al., (2010). Tags were set to record tri-axial acceleration at 5 Hz for ~ 4 days.

Whale sharks (*Rhincodon typus*) were caught between April and June 2007 to 2009 at Ningaloo reef, Australia. Sharks were equipped with daily diaries which contain a tri-axial accelerometer, tri-axial magnetometer, depth, temperature, speed, light, humidity and GPS sensor (Wildlife Computers, Washington, USA) (Wilson et al., 2008). Tags were positioned on the second dorsal (Fig.3.1iii) using a clamp to secure the tags in place. For full methods and tag attachment see (Gleiss et al., 2009). Tags were set to record at 5 Hz.

For all animal-borne tags, accelerometers were set to record tri-axial acceleration continuously, corresponding to the surge, heave and sway of the fish (Fig.3.1i, Table 3.1).

Table 3.1. Individual characteristics for study animals

Species	ID	Length (cm)	Weight (g)	Sex	Recording frequency (Hz)
Trout	A09303	36.4	660	F	30
	A09302	37.0	910	F	30
	A09298	38.0	850	F	30
	A09296	40.2	940	F	30
	A09301	41.2	1080	F	30
	A09297	43.1	1060	F	30
Bass	A07112	51.0	2710	F	30
	A07106	53.5	1600	F	30
	A07108	54.2	2000	F	30
	A07104	55.0	2800	F	30
	A07105	53.0		M	30
Eel	A07109	65.7	678	F	30
	A07950	56.6	431	F	30
	A07955	63.4	629	F	30
	A07108	69.3	820	F	30
	A07111	75.7	820	F	30
	A07097	84.2	930	F	30
	A07956	81.0	908	F	30
Cod	A07100	59.2	2486	F	25
	A07957	77.0	5576	F	20
	A07099	59.1	1998	M	25
	A07973	67.4	4729	F	20
	A07974	78.1	6996	F	20
	A07960	78.2	5887	F	20
	A07958	84.3	9010	F	20
Whale shark	Cet	450.0	-	M	5
	Por	500.0	-	M	5
	Rhi	700.0	-	M	5
	Meg	800.0	-	M	5
Nurse shark	D	154.0	-	M	5
	M	211.0	-	M	5
Blacktip shark	A05468	149.0	-	F	30

Fish Simulation

A fish swimming simulation was used to identify the best method for calculating the optimal smoothing frequency. For accelerometer-tagged fish, we cannot directly identify the proportion of total acceleration partitioned between static and dynamic motion. A simulation was therefore used to mimic the static and dynamic acceleration of a steadily swimming fish, which, in turn, was used to

identify a method for accurately disentangling the two components in real, fish-derived data.

We simulated the swaying acceleration (Fig. 3.2) of a fish swimming using a common mode of propulsion (similar to carangiform/ subcarangiform/ anguilliform propulsion) because the vast majority of dynamic acceleration in swimming fish (with the exception of flatfish) occurs in the lateral plane, supporting our single axis simulation approach. In addition, even simulation of flatfish swimming would only necessitate a change in the axis under consideration. The swaying acceleration includes regular peaks corresponding to the tail beat frequency (TBF) and tail beat amplitude (TBA), which are important metrics for estimating locomotor activity of fish (Herskin and Steffensen, 1998; Kawabe et al., 2003; Steinhausen et al., 2005).

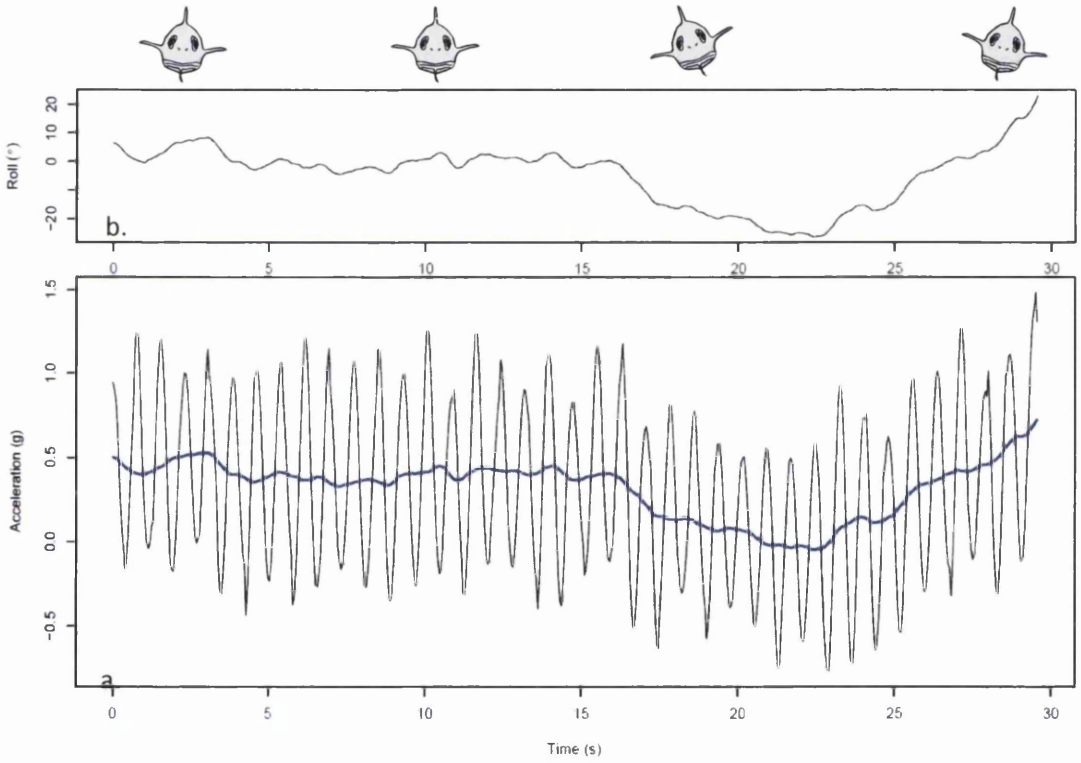

Figure 3.2. Example of a fish simulation, showing (a) the constructed static acceleration (solid blue line) and dynamic acceleration combined, and (b) the equivalent roll angle.

To mimic the oscillations in the sway axis, the simulation was designed to have a variable TBF and TBA (Dy_{sim}; Fig. 3.2a), as is commonly encountered in freely swimming fish (Hunter and Zweifel, 1971; Steinhausen et al., 2005). In addition, changes in roll (stability) may necessitate the inclusion of changes in the static acceleration (St_{sim}). This static component was simulated using a correlated random walk with boundaries set at -1 and +1 g, representing a roll of 0° to ± 180 °, i.e. level to upside-down. The dynamic and static components were combined ($Acc_{sim} = Dy_{sim} + St_{sim}$) providing a complete simulation of the factors contributing to measured swaying acceleration of a level swimming fish (Fig. 3.2a).

To separate the Dy_{sim} from the St_{sim} a range of symmetrical running means (0.1 to 10 seconds) were used on the fish simulation trace (Acc_{sim}):

$$St_{pred} = \sum_{i=-n}^{n} w_i \, x_{t+i} \tag{1}$$

where, x_t is the original time series, w_i and $i = -n$ are the weights (representing smoothing terms from 0.1 to 10 seconds) and St_{pred} is the smoothed raw dataset (static acceleration). The St_{pred} was used to derive the corresponding Dy_{pred}, which was calculated as $Acc_{sim} - St_{pred}$.

The optimal smoothing algorithm was calculated for each simulation using time series of the smoothed static acceleration (St_{pred}) and the simulated static acceleration (St_{sim}):

$$Opt_{sim} = min\left(\sum |St_{sim} - St_{pred}|\right) \tag{2}$$

Thus, Opt_{sim} represents the smoothing term (in seconds) that minimises the sum of the difference between the simulated acceleration (St_{sim}) and that of the smoothing algorithm generated static acceleration (St_{pred}), i.e. the smoothing option representing the closest agreement between simulated and estimated data.

For datasets obtained from live fish, the static acceleration (St_{sim}) is unknown and must be estimated using smoothing algorithms with unknown parameters. To identify a reproducible method by which the static acceleration could be derived, we utilised our simulation method to identify statistical markers of the optimal smoothing window size. Specifically, we extracted a range of statistical properties (Table 3.2) of the derived static and dynamic acceleration from a wide range (0.1-10 s) of smoothing parameters. These statistical properties were then evaluated for minima or maxima in the range simulated to indicate obvious "markers". We then used 100 simulations and repeated the above analysis to evaluate consistencies in all potential markers. The metric (either singularly or in combination) which produced optima closest to Opt_{sim} (least difference and least variation) was selected as the parameter for use on non-simulated fish datasets.

Table 3.2. Definitions of statistical markers used to estimate the optimal smoothing term.

Acronym	Statistical parameter of dynamic and static acceleration
CV	Coefficient of variation
K	Kurtosis
M	Mean
U	Upper (max)
R	Range
D	Standard deviation
E	Standard error
S	Skew
V	Variance
P	Proportion of spectrum attributed to static
KP	Kurtosis and proportion attributed to static

Acceleration Processing and Statistical Analysis

The static component of the raw acceleration was calculated using the same method as described in the fish simulation section, with running means of total acceleration over periods of 0.1 to 10 seconds. The number of data points for each mean varied depending on the species, in accordance with the recording frequency. Twenty-five blocks of thirty second steady swimming were extracted for each individual. The technique for identifying the optimal smoothing algorithm (calculated from the simulation) was applied to each thirty second block of steady swimming providing an estimate for the optimal smoothing window for each swimming bout.

For each swimming bout, TBFs were calculated by identifying the number of peaks in the sway acceleration. A linear mixed effects model was used to identify the interaction between fish size or TBF and optimal smoothing interval, with TL and sex set as fixed variables and species as a random factor. All averages are as mean ± SE and the statistical significance level (α) was set at 0.05. All statistical analyses were conducted in R (v. 2.15.3 The R Foundation for Statistical Computing, Vienna, Austria), using the lme4 package (Bates, Maechler & Bolker, 2012).

Results

Methods for deriving the optimal smoothing interval from fish simulations

When markers to predict the optima were used alone, the optimum smoothing algorithm was over- and under-estimated with a high standard error (Fig. 3.3), notably dynamic acceleration markers produced estimates closer to the optima than static markers (Fig.3.3).

Markers were therefore used together to identify a more robust and reproducible optima. The marker which produced estimates closest to Opt_{pred} (and with the least variance) utilised the kurtosis of the dynamic acceleration and the power spectrum of the static acceleration (Fig.3.3).

Difference between the optima derived by statistical markers (Opt_{pred}) and the optimal sampling frequency (Opt_{sim}) using simulated datasets. See table 3.2 for a key showing the definitions of statistical parameters.

Firstly, the minimum kurtosis of the dynamic acceleration ($min\beta_2$) was used as an initial estimate of Opt_{pred}:

$$\beta_2 = \left(\frac{\sum (Z_{Dyn} - \mu)^4}{\left(\sum (Z_{Dyn} - \mu)^2 \right)^2} \right) - 3 \quad (3)$$

where, μ is the mean and Z_{Dyn} is the dynamic sway acceleration. Kurtosis (β_2) measures the extent to which a distribution is peaked (DeCarlo, 1997). Under-

smoothed acceleration results in a relatively high β_2 value (Fig. 3.4c), which then decreases as the signal from the tail beat is reduced over longer smoothing windows and the distribution of the Dy_{pred} becomes more uniform (DeCarlo, 1997) (Fig.3.4d).

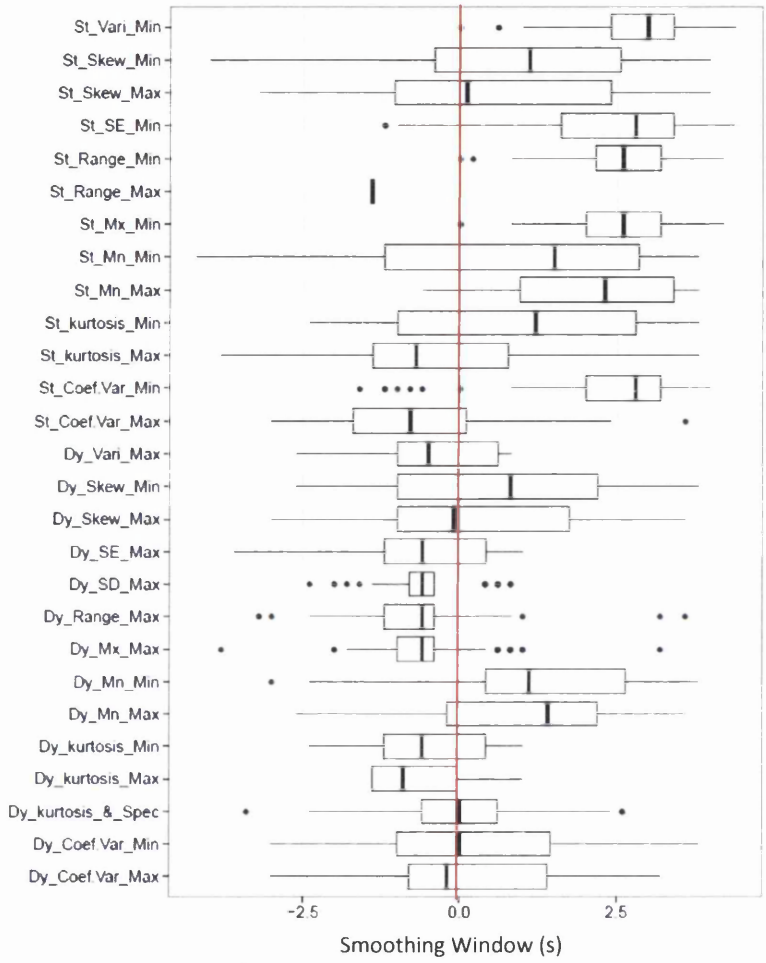

Figure 3.3. Performance of markers to estimate the optimum smoothing window from simulated data. The red line = the line of equality, with values closer to 0 representing markers predicting accurate and precise values.

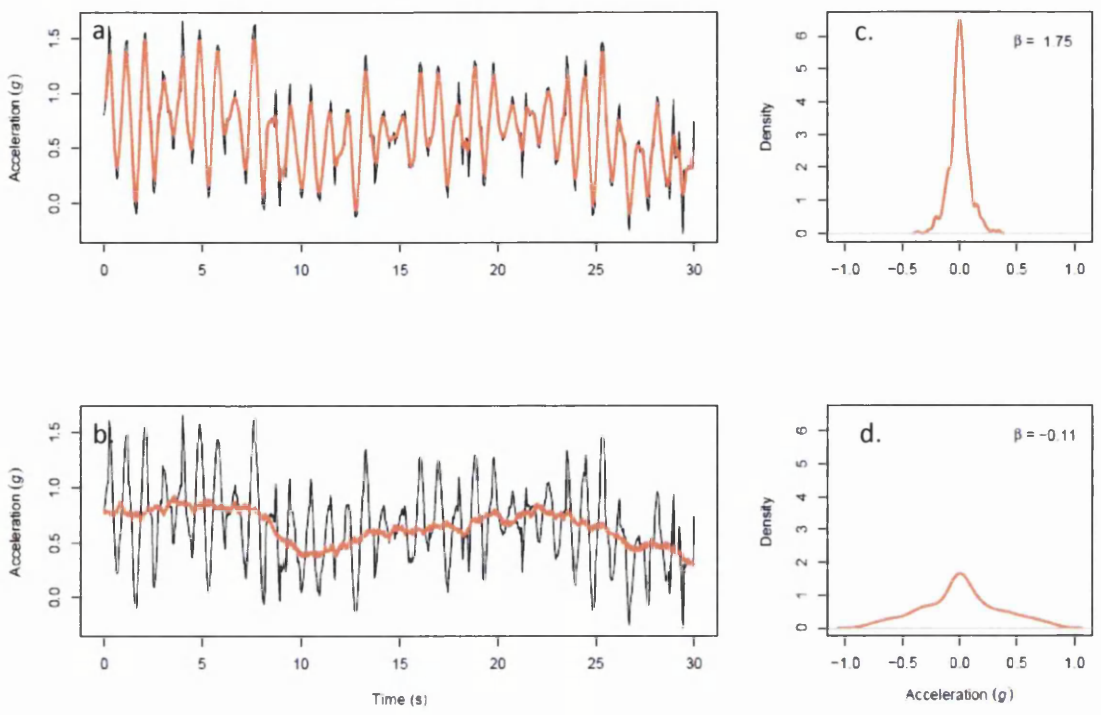

Figure 3.4. Example of a fish simulation for smoothing algorithms which correspond to under (a) and optimally (b) smoothed raw acceleration (Acc_{sim}), with the corresponding Dy_{pred} distribution for under and optimally smoothed data (c, d, respectively). Acc_{sim} is the solid black line and St_{pred} is the solid orange line. The corresponding value for the kurtosis of the $Dy_{pred}(\beta_2)$ are shown in the upper right corner of the plots aii and bii.

When estimates were made using the minimum kurtosis of the dynamic acceleration alone, smoothing length was often underestimated (Fig.3.3). Estimates were therefore corrected using the power spectrum of the static acceleration to identify the proportion attributable to static (St_{pred}) and dynamic (Dy_{pred}) motion. The power spectrum of St_{pred}, Dy_{pred} and Acc_{pred} provides the location of the dominant acceleration signal for the static, dynamic and combined acceleration, respectively (Fig. 3.5). The static power spectrum peaks at a lower frequency (Fig.3.5aii) relative to the dynamic power spectrum (Fig.3.5bii). Thus, when the static and dynamic components are combined (Acc_{pred}) there are two spectral peaks which correspond to the lower and higher frequencies of the static and dynamic acceleration (Fig.3.5cii).

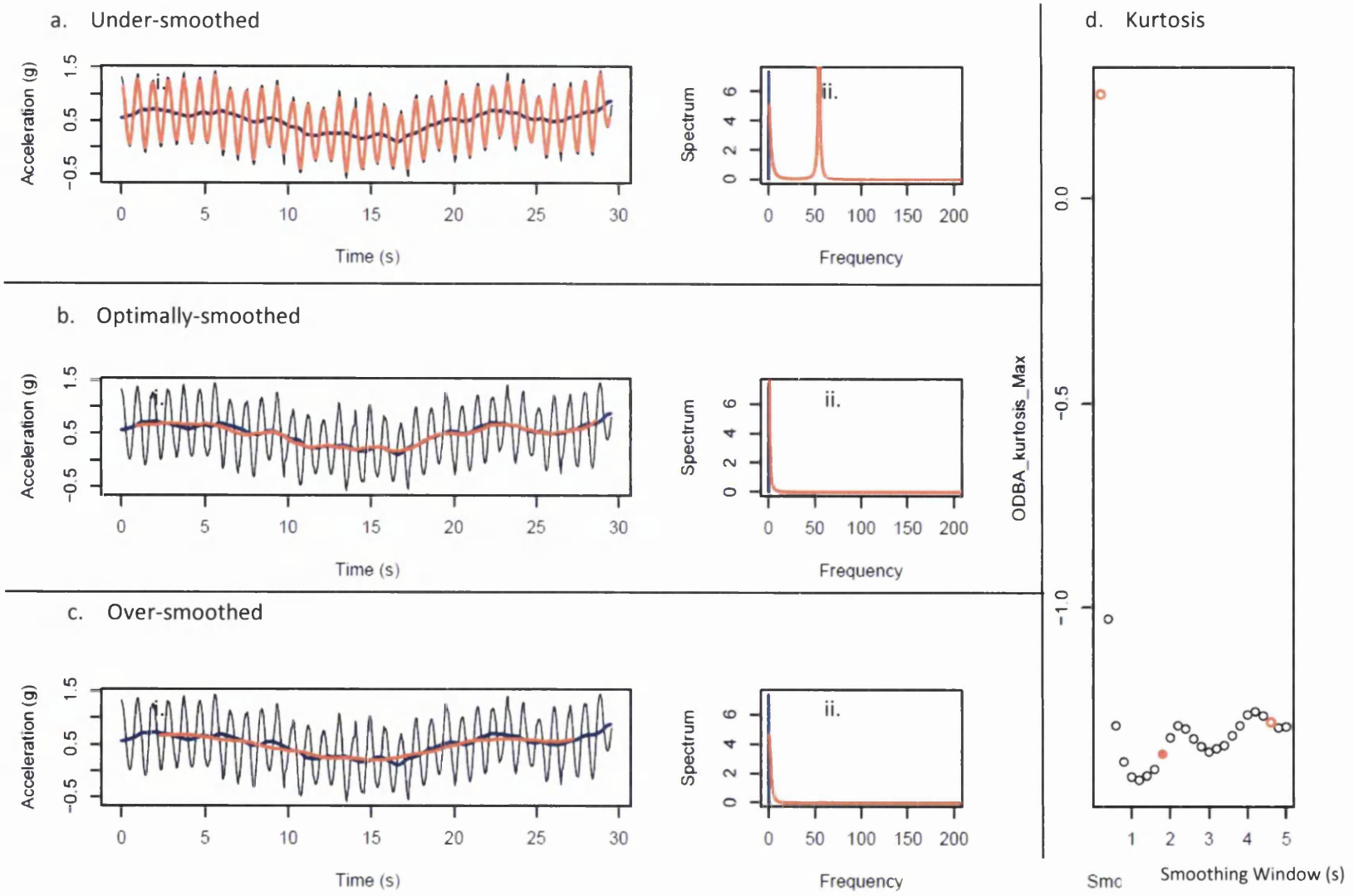

Figure 3.5. Example of a fish simulation for smoothing algorithms which correspond to under (ai), optimal (bi) and over (ci) smoothed raw acceleration (Acc_{sim}) and the corresponding power spectrum (aii, bii, cii), respectively. Acc_{sim} is the solid black line, St_{sim} is the solid blue line and St_{pred} is the solid orange line. The position of the running mean lengths relative to the dynamic kurtosis (d) is shown for optimally smoothed (filled orange points) and over and under smoothed data (hollow orange points before and after the optima).

When the smoothing window was lower than the optimum, the static acceleration was defined as under-smoothed, which results in an over-representation of the dynamic acceleration in the static spectrum (Fig.3.5aii). When the smoothing window was higher than the optimum, the static acceleration was over-smoothed, which results in the mis-representation of both the static acceleration and dynamic acceleration (Fig.3.5cii). The first smoothing term after the minimum kurtosis marker which ≥ 90 % of the derived static acceleration spectrum was attributed solely to static acceleration was defined as the optimal smoothing algorithm.

Optimal Smoothing Interval of empirical data

The optimal smoothing algorithm for the data derived from animal-borne tags was estimated using the method described above (see supplementary 2 for code used to estimate optima's). Optimal smoothing windows ranged from 1.71 seconds for rainbow trout to 6.35 seconds for whale shark (Table 3.3). See figure 3.6 for an example of the raw dynamic acceleration of a European sea bass with the estimated optimal smoothing term.

Table 3.3. Species-dependent optimal smoothing interval ($Interval_{opt}$) for steadily swimming fish. The number of fish is shown by N_{Fish} and the number of swimming bouts extracted for all fish is shown by N_{Run}

Species	N_{Fish}	N_{Run}	$Interval_{opt}$ (s)			TBF (Hz) ($\bar{x} \pm SE$)
			$\bar{x} \pm SE$	Minimum	Maximum	
Sea bass	5	24	1.79 ± 0.17	0.80	4.00	0.98 ±0.08
Atlantic cod	7	27	2.21 ± 0.18	0.60	4.00	0.51 ± 0.02
European eel	7	25	1.90 ± 0.22	1.00	6.00	0.92 ± 0.04
Rainbow trout	6	38	1.71 ± 0.07	1.00	2.40	0.88 ± 0.02
Blacktip shark	1	1	5.20 ± 0.00	5.20	5.20	0.72 ± 0.00
Nurse shark	2	27	2.47 ± 0.34	0.40	8.60	0.12 ± 0.01
Whale shark	4	37	6.35 ± 0.26	3.20	9.00	0.20 ± 0.01

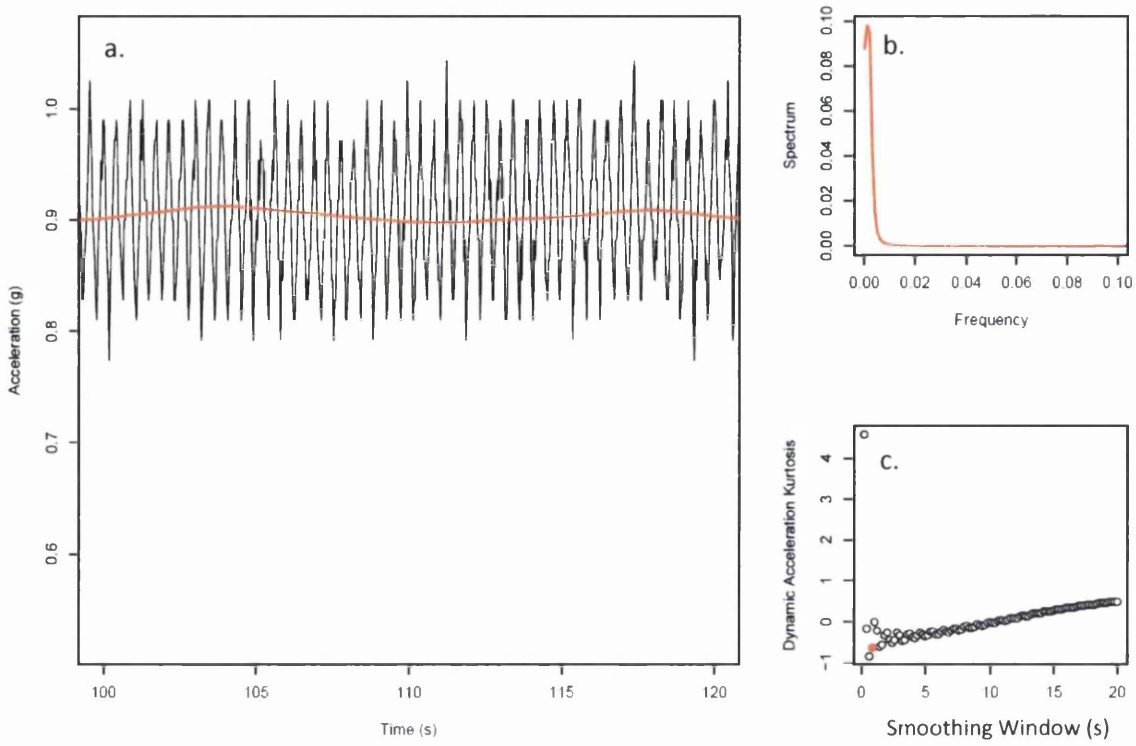

Figure 3.6. Position of optimal smoothing algorithm for a European sea bass, *Dicentrarchus labrax* showing (a) raw dynamic sway acceleration (black line) with the optimal smoother (orange line), (b) the power spectrum of the optima and (c) the dynamic acceleration kurtosis in relation to the smoothing frequency. The optima is highlighted as the orange point.

When fish size was regressed against the optima, length was the only significant (P=0.015) predictor variable with a positive effect on the optimal smoothing interval (Fig.3.7a). Sex was non-significant (P=0.926):

$$\log(Int_{Opt}) = 0.392 \cdot \log(TL) - 0.986 \quad (4)$$

where, TL is in (cm).

The average TBF for each species varied from 0.12 Hz for nurse shark to 0.98 Hz for sea bass (Table 3.3). The relationship between TBF and the optimal smoothing algorithm was developed using a mixed model. TBF was the only significant predictor variable (P 0.002) with a negative effect on the optimal smoothing interval (Fig.3.7b):

$$\log(Int_{Opt}) = -0.356 \cdot \log(TBF) + 0.583 \quad (5)$$

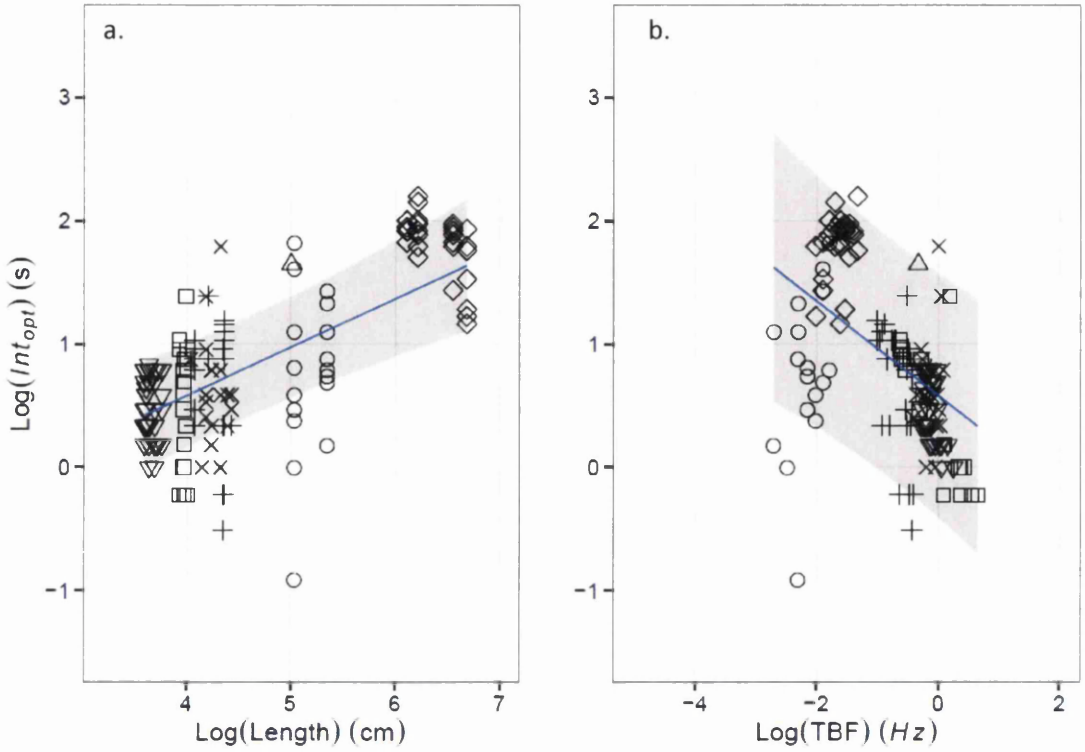

Figure 3.7. Log-log relationship between optimal smoothing interval (Int_{opt}) and fish length (a.) and tail beat frequency (TBF) (b.) for European sea bass (□), Atlantic cod (+), European eel (×), rainbow trout (▽), blacktip shark (△), nurse shark (○) and whale shark (◇). The regression lines correspond to fits from mixed effects models (a; $\log(Int_{Opt}) = 0.392 \cdot \log(TL) - 0.986$ and b; $\log(Int_{Opt}) = -0.356 \cdot \log(TBF) + 0.583$). The confidence intervals of the fit are shown by the shaded regions.

Discussion

The present study provides an automated tool for separating static and dynamic acceleration for fish. We also highlight the value of using simulations to help validate techniques for processing data from animals tagged with accelerometers. For fish between 0.36 – 8 m length the metric that provides the optimal smoothing algorithm closest to Opt_{pred} (and with the least variance) is the kurtosis of the dynamic acceleration and the power spectrum of the static acceleration.

Simulation

To accurately separate the static acceleration from the dynamic part in a steadily swimming fish, the acceleration must be averaged over a period greater than one stroke cycle (tail-beat). TBF and the duration of a stroke cycle is size specific with larger organisms having a lower TBF (Bainbridge, 1958; Sato et al., 2007; Shepard et al., 2008). Results from this study concur with this, as evidenced by the negative relationship between TBF and optimal smoothing algorithm length and, conversely, the positive relationship between TL and smoothing algorithm. We note that estimates of optimal smoothing terms are relatively similar to previously calculated smoothing terms for both sea bass (1.79 compared to 2.00 in (Wright et al., 2014)), and whale sharks (6.35 compared to 5 in Gleiss et al. (2013)). However TBF estimates of nurse sharks in the present study are lower than estimated in previous studies (Whitney et al., 2010). The disparity between TBF estimates are not unrealistic as the TBF may vary depending on the behaviour of the fish, further highlighting the importance of adapting the smoothing interval both within and between species.

Potential errors

The location of the accelerometer vary between species, which will introduce variability in the amplitude of the swaying acceleration (tail-beat amplitude). However, this variability will not necessarily affect the optimal sampling frequency as the dominant influence on the optimal sampling frequency is the TBF, which remains constant independent of tag location. It must also be noted that for larger species, like whale shark, it may be more difficult to separate the dynamic from the static acceleration, as postural shifts (static acceleration) can also occur during steady swimming.

Optimal smoothing algorithms are inevitably depend on the level of activity, through changes in tail-beat frequency exhibited by the fish. As such, no single smoothing window will perform optimally over all potential activity levels exhibited by an individual. However, during the range of exercise that comprises routine activity, this variability is likely very low. For instance, in cod, routine activity

comprises 0.37 Hz to 0.65 Hz in tail-beat frequency and for this range a single smoothing solution will perform adequately. According to our tail-beat frequency scaling relationship, the entire range of activities exhibited by a steadily swimming cod would result in difference of 0.46 seconds in the optimal smoothing algorithm. The error inherent in this minor misrepresentation of static acceleration is therefore likely inconsequential for any conclusions drawn from the data.

Conclusion

Techniques to process and analyse acceleration data are of increasing importance, as accelerometers are ever-frequently used in the study of animal behaviour in the laboratory (Wright et al., 2014) and field (Bom et al., 2014; Wilson et al., 2014). The variability in the optimal smoothing length for different species (Table 3.3) shows the importance of adapting smoothing terms both within and between species. Within species, variation in optimal smoothing algorithm can create a problem for researchers wishing to separate dynamic from static acceleration in long term studies, as noted by Shepard et al., (2008). The method used in this paper can be applied to fish swimming at different speeds by adapting smoothing terms within a recorded acceleration track, thereby improving the accuracy of extracted dynamic activity levels.

The present study highlights differences in optimal smoothing algorithms for steadily swimming fish. Future studies should address the effect of smoothing on fish swimming using different modes of propulsion, with a focus on more complex behavioural events like fast-starts. This will likely have a large effect on the separation of the dynamic and static acceleration.

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Chapter 4

Estimating activity-specific energy expenditure in teleost fish, using accelerometer loggers: *Aerobic Activity*

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S. R Wright was responsible for data collection, analysis and writing the manuscript. All authors proof read and made amendments to the manuscript. Additional data from rainbow trout and European eel has been added to the published manuscript to expand the scope of the study.

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Abstract

The ability to define and quantify the behaviour and energetic costs of different activities is fundamental to a full understanding of fish ecology and movement, but monitoring activity and measuring energy expenditure in fish in the field is problematic. New telemetry methods using data loggers that incorporate tri-axial accelerometers promise to provide a method for simultaneously recording the behaviour and activity-specific energy use in both the laboratory and field. Using electronic data loggers equipped with tri-axial accelerometers we have measured dynamic body acceleration (DBA) during aerobic exercise in European sea bass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*) whilst swimming in a swim-tunnel respirometer. Experiments with bass were also used to assess the effect of changes in ambient water temperature between 5.5 and 17.5 °C. For all individuals, dynamic body acceleration (vectorial dynamic body acceleration [VeDBA] or overall dynamic body acceleration [ODBA]) scaled linearly with oxygen consumption and as a function of ambient temperature. When the two DBA metrics were compared, VeDBA was not significantly different from ODBA, though the value for Akaike's information criterion was lower for VeDBA (indicating a better fit for the VeDBA model). In this paper we provide further evidence to support the use of acceleration as a means to quantify the activity-specific energetic costs of swimming in teleosts and highlight some of the problems associated with monitoring the activity and metabolic rate of fish in restricted laboratory conditions.

Introduction

It has been proposed that metabolic rate can be considered a 'universal currency' in animal biology and ecology, providing an objective measure that can be used in attributing 'cost' to different activities and to assess what animals do to achieve energetically optimal behaviour (Brown et al., 2004; Shepard et al., 2009). Trade-offs between the cost and benefit of specific behaviours govern the success and survival of animals through natural selection. For example, for many species of fish the choice of being active or inactive at different times of the day will affect both feeding success (Bestley et al., 2008; Murphy et al., 2011) and the risk of predation (Béguer-Pon et al., 2012; Stuart-Smith et al., 2007). Measuring the energy turnover and ways in which energy is allocated to specific activities is therefore of central importance in our understanding of behavioural and physiological ecology.

Accurate estimates of metabolic rates can be made, relatively easily, in the laboratory, either directly by measuring heat output (calorimetry) (Pakkasmaa et al., 2006; Penttinen and Kukkonen, 2006; Walsberg and Hoffman, 2005) or indirectly by measuring oxygen consumption, M_{O_2}(Clarke and Johnston, 1999). However, measuring field metabolic rate (FMR) is more problematic, especially for aquatic animals like fish. Presently, methods used to estimate FMR involve the indirect measurement of oxygen consumption, for which approaches include the use of Electromyogram (EMG) telemetry (Cooke et al., 2004; Hinch et al., 1998), doubly labelled water (DLW) (e.g. (Shaffer, 2011)), or records of heart rate (f_H) (e.g. (Gilman and Wells, 1993). EMG telemetry involves measuring bioelectrical voltage changes in the muscle of the subject and has provided great insights into the activity and behaviour of free-ranging animals, including fish (Hinch et al., 1998). DLW measures the subject's carbon dioxide production by monitoring relative changes in stable isotopes of oxygen and hydrogen (Speakman, 1998). DLW can be used for air breathers, including mammals (Nagy, 2005), reptiles (Bennett and Nagy, 1977) and birds (Masman et al., 1988; Mullers et al., 2009; Shaffer et al., 2001), but not for fish, because the high water flux across the gills and the low metabolic rate result in only slight changes in the divergence of isotope concentrations (Speakman, 1997). In contrast, f_H has been shown to provide a reasonably useful estimate of metabolic rate in large (>1 kg) teleosts (Casselman et al., 2012; Clark et al., 2005), although

limitations include high equipment costs, poor reliability (Butler et al., 2004) and variable relationships between f_H and oxygen consumption (Iversen et al., 2010; Thorarensen et al., 2005). For example, 'frequency modulators' (like sea bass *Dicentrarchus labrax*) are able to regulate their cardiac output during exercise and digestion, causing differences in the scaling of f_H with oxygen consumption (Iversen et al., 2010). Both the f_H and DLW methods involve uncertainties; so, for fish, these methods do not fully provide behavioural and physiological ecologists with comprehensive tools for the study of FMR.

Movement is one of the four main bodily functions which incur energetic costs in ectotherms, the others being basal metabolic rate (BMR) (Frappell and Butler, 2004), temperature-dependent energy expenditure (Beamish and Trippel, 1990) and specific dynamic action (SDA) (McCue, 2006). These latter 3 are perhaps most easily modelled since estimates are accessible via allometric relationships, and they vary less in time and space than do movement-associated costs (cf. (Claireaux et al., 2006; Fitzgibbon et al., 2007; Jordan and Steffensen, 2007). Movement-induced energetic expenditure is governed by muscle contractions and is typified by variable acceleration in the body (Gleiss et al., 2010), so records of the (3-dimensional) acceleration of fish should provide a useful proxy for activity-specific energy expenditure. Indeed, based on this, recent studies have already correlated dynamic body acceleration (DBA—derived from tri-axial acceleration measurements) with oxygen consumption for a range of terrestrial and aquatic species, including cane toads (Halsey and White, 2010), cormorants (Wilson et al., 2006) and hammerhead sharks (Gleiss et al., 2010). Bi-axial acceleration (Clark et al., 2010) and acoustically transmitted acceleration (Murchie et al., 2011; Wilson et al., 2013) have also provided some exciting insights into fish behaviour and physiology. However, more studies are required to improve methodologies in this field of research, with issues arising from the complex interpretation of raw acceleration.

In this study, we have attempted to identify how DBA scales with activity and oxygen consumption for three species of teleost fish, European sea bass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*), in order to establish the extent to which DBA may provide a useful proxy for active metabolic rate (metabolism induced by movement of the body) as it has been shown for other animals. The effects of tagging, temperature and

scaling of DBA derived using different data-processing methods are also explored and compared for sea bass.

Materials and Methods

Experiments for sea bass and rainbow trout were regulated by the UK Animals (Scientific Procedures) Act 1986 and carried out under the authority of UK Home Office project licence PPL 80/2434, having been approved by Cefas' local ethical review process. Experiments with European eel complied with Dutch law on animal experiments and were approved by the animal ethical committee of Leiden University (DEC#10231).

Approach

To establish and quantify the relationship between DBA and metabolic rate, we simultaneously recorded DBA and mass-specific oxygen consumption (M_{O_2}). DBA was recorded using externally (European eel) or internally (sea bass and rainbow trout) implanted loggers which were equipped with 3-dimensional accelerometers. Oxygen consumption was measured in a swim-tunnel respirometer at rest and during steady state, fully aerobic swimming, at speeds between 0.3 and 1.8 body lengths (BL) s^{-1}. During swim trials, up to 3 measurements of M_{O_2} were recorded at each water speed, depending on the swimming capabilities of each fish.

Animals

1. Rainbow trout

Aquaculture reared rainbow trout, *Oncorhynchus mykiss*, (n=6) were obtained from a commercial farm and were kept at Cefas Laboratory, Lowestoft at ambient water temperatures. Fish were fed commercial trout pellets every 1 to 2 days. Total lengths ranged from 37.0 to 44.5 cm, with total weights from 634 to 1066 g.

2. *European eel*

Aquaculture reared European eel, *Anguilla anguilla*, (n=9) were obtained from the Passie voor Vis B.V., Sevenum, Netherlands. Experiments took place at the University of Leiden, Holland, where eels were kept in an aerated tank of fresh water (7000l). Temperatures were maintained at 18±2 °C with a photoperiod of 12/12, light/dark. Total lengths ranged from 56.6 to 81.0 cm, with total weights from 431 to 908 g.

3. *Sea bass*

Bass (n=9) were obtained from Sea Life Centre at Great Yarmouth, Norfolk, UK, and held at the Cefas Laboratory, Lowestoft, in a tank supplied with aerated seawater at ambient temperatures (5.2 to 17.8 °C). Total fish lengths ranged from 45.1 to 55.0 cm, with total weights from 1100 to 2200 g. Bass become easily agitated when attempting to transfer them between tanks; thus, to acclimatise them to the presence of the net and to reduce the stress when moving them from the holding aquaria to the swim-tunnel, we exposed them to a daily 'net challenge' by placing the net in their tank for 2min each day, after which they were fed a diet of sand eel or sardine (*ad libitum*).

Experimental setup

Respirometry

1. *Sea bass and trout*

For sea bass and trout, oxygen consumption was measured in a Brett-type swim tunnel respirometer (Brett, 1965) using intermittent flow respirometry as described by Melzner et al., (2009). Briefly, the respirometer (swim chamber section of 25 × 25 × 87 cm) was submerged in an outer tank, which measured 232 × 95 × 70 cm having a total water capacity of 187 l (Loligo Systems, ApS). The outer tank served as a source of aerated water used for flushing (flush pump, Eheim, 20l min^{-1}) the respirometer (swim chamber) after each 'closed' measuring phase. Water quality

in the outer tank was maintained by providing an inflow (10l min^{-1}) of fresh seawater. The water in the outer tank was kept fully aerated, and temperature was maintained using a temperature regulator (Loligo Systems). Prior to each experiment, water velocities in the swim chamber were measured using a flow meter (HFA, Höntzsch) to calibrate the water speed control. Each swim trial was broken down into 'measurement', 'flush' and 'wait' phases. During the measurement phase, the oxygen tension of the water in the swim chamber was recorded using a galvanic oxygen electrode, while the swim chamber was completely closed from the outer tank. Oxygen consumption was calculated (see next paragraph) from the rate of decrease in oxygen tension. Subsequently, the swim chamber was flushed with aerated seawater from the outer tank to replenish oxygen levels (flush phase), and then a 'wait' phase enabled the oxygen levels to stabilise before the next measurement phase.

During swim trials, chamber flushing and the recirculation valve were controlled through an interface (DAQPAC- G1X, Loligo Systems) connected to a PC running AutoResp™ software (Version 1.6, Loligo Systems). Oxygen tension within the swim chamber was measured using a mini-DO galvanic cell oxygen probe suspended into the water current of the respirometer, which was connected to the DAQ interface, and oxygen saturation data was calculated using Auto - Resp™. To encourage the fish to maintain position toward the front of the swim chamber, a visual cue for orientation (Griffiths and Alderdice, 1972) was created using strips of black electrical tape spaced 1cm apart, fixed vertically to the outer wall at the front of the chamber. After each experiment and after the fish had been removed from the swim chamber, oxygen saturation was monitored for a further 6h to measure any oxygen consumption resulting from bacterial contamination. To identify whether tagging had any effect on the oxygen consumption, swim trials were conducted on fish both before and after logger implantation.

2. *European eel*

For eel experiments, oxygen consumption was measured in a Blazka-style swim tunnel respirometer, as described in Thillart, (2004). The respirometer had a total water capacity of 127±1.09 l, with a 190 mm diameter inner tube swimming section. The surface area of the inner tube is equal to the surface area of the outer tube (after correction for wall thickness), such that water velocity is the same in both compartments. To alter flow speed, the swim tunnel has a three-phase electromotor with a power output of 400W (Siemens Micro Master: Basic 370) which drives a propeller consisting of three blades. At the front and end of the swim tunnel are flow stabilisers (10 mm spacing), leaving a 1300 mm swimming section. To measure the oxygen use of eels at different flow speeds, intermittent flow-through respirometry was used, as described for trout and bass experiments. Oxygen electros (Mettle Toledo, Germany) measured the decline in oxygen level over time. Oxygen levels did not fall below 75% saturation.

Accelerometer data-loggers

Acceleration was recorded using G6a electronic data loggers (Cefas Technology; dimensions: 40 mm × 28 mm × 16.3 mm, 18.5 g in air, 6.7 g in seawater). Loggers were pre-programmed to record acceleration data at 30 Hz and temperature once a minute for specific periods between the end of the surgical recovery and the end of the study. Acceleration was recorded in 3 planes of motion (Fig.4.1), the X (dorso-ventral: heave), Y (side to side: sway) and Z (anterior-posterior: surge). To maximise logger life, the data logging regime was designed to record principally during the swim trials. However, for experiments with sea bass, to compare the swimming activity during swim trials with their spontaneous swimming activity, a number of additional recording periods were included in the data logging regime to allow data to be gathered during periods when bass were swimming with conspecifics in their holding tank.

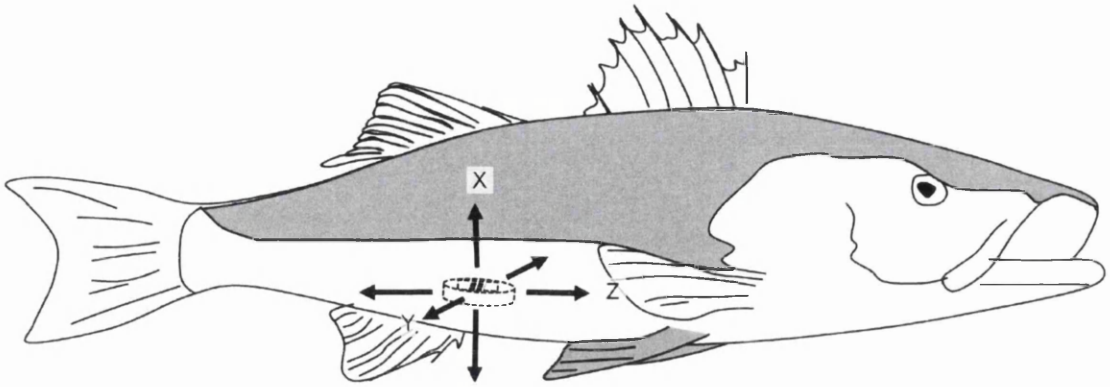

Figure 4.1. Schematic diagram of a European seabass (*Dicentrarchus labrax*) internally implanted with a Cefas G6a data-storage tag. Arrows indicate the (X) heave, (Y) sway and (Z) surge axes of acceleration recorded by the logger.

Surgical Implantation

1. Trout and Bass

For experiments with trout and bass, loggers were implanted surgically into the peritoneum of the fish. Prior to surgery, fish were anaesthetised by immersion in (sea or fresh) water containing 2-phenoxyethanol (4ml l^{-1}) for 3 to 5 min. As fish lost equilibrium and opercula beat slowed, fish were measured and weighed. Fish were then placed on an operating table and held immobile in a sponge block. During surgery the gills were continuously irrigated with aerated seawater containing a maintenance dose (2ml l^{-1}) of anaesthetic (as above). A 3 cm midline ventral incision was made through the skin and muscle 4 cm anterior to the cloaca. The sterilised logger was then inserted into the peritoneal cavity and was sutured to the peritoneal wall to prevent it from moving within the peritoneum during the study and to decrease the risk of the logger being expelled through the incision wound (cf. (Moore et al., 1990)). The incision was closed with 2 or 3 interrupted sutures, and the wound was dressed with antibiotic powder (Vetremox antibiotic powder with an Orahesive mix [50:50]). Fish were then moved to their holding tank and held in a stream of fresh, aerated seawater until they regained equilibrium and strong opercular movements had resumed. The surgical procedure, from initial anaesthesia to final incision closure, took no more than 17min. Fish were allowed a minimum of 4 weeks to recover and to allow the data logger to become en capsulated (and therefore stabilised) within the body cavity, before swim trials were conducted.

2. European eel

For experiments with eel, loggers were attached on the midline of the dorsal surface behind the pectoral fins. Prior to tag attachment, fish were immersed in freshwater containing clove oil. As fish lost equilibrium and opercular beat slowed, fish were measured and weighed, before they were placed in the centre of a water soaked towel with rolled edges. A thin layer of suede was attached to the underside of the logger to reduce friction between the logger and the skin surface of the fish. Loggers were then sutured subcutaneously through the suede and skin in four locations (four corners of the suede) before eels were placed in the swim flume for recovery overnight (minimum of 12 hours).

Swim trials

Experiments with trout and eel solely focused on assessing whether there was a significant correlation between acceleration and M_{O_2}. Studies with bass also identified whether scaling relationships were affected by ambient water temperature, as temperature affects the metabolism of ectothermic teleosts (e.g. (Claireaux et al., 2006) add). Bass experiments were carried out at ambient seawater temperatures between June and September in 2011 (13 °C to 14 °C) and then between February and September in 2012 (5 °C to 18 °C) to encompass a range of seasonal water temperatures between 5 °C and 18°C. Although the plan was that each bass would be swum during the summer and winter trials, 3 fish expelled their data loggers before the end of the study (cf. (Moore et al., 1990)). Consequently, only data from the remaining bass were used for further swim trials; resulting in fewer fish in certain temperature groups (Table 4.1).

Table 4.1 Experimental temperature groups for European sea bass, including the number of fish (n) the individuals (Fish ID) and the fish characteristics for each group

Temperature Group	n	Mean Temperature ± S.D. (°C)	Fish ID	Mean TL ± S.D. (cm)	Mean Weight ± S.D. (g)
6	4	6.05 ± 0.32	4, 6, 8, 9	52.42 ± 2.2	2435 ± 195
7	1	7.27 ± 0.13	7	55.00 ± 0.0	2200 ± 0
8	3	8.06 ± 0.35	5, 7, 8	52.22 ± 1.5	1667 ± 380
13	2	12.68 ± 0.03	1, 3	49.91 ± 3.8	2025 ± 692
14	3	13.71 ± 0.12	1, 2, 3	50.87 ± 3.2	2145 ± 587
17	3	17.27 ± 0.16	7, 8, 9	52.13 ± 2.1	2749 ± 32

To remove any effects of specific dynamic action on oxygen consumption after feeding (Axelsson et al., 2002; Dupont-Prinet et al., 2009), fish were fasted for 72 h prior to swim trials. They were then measured, weighed, placed in the swim tunnel and allowed a minimum of 12 h to recover from the oxygen debt accumulated during capture and transfer (Melzner et al., 2009; Schurmann and Steffensen, 1997). During acclimation, water speed was held constant at between 0.3 and 0.4 BL s^{-1}.

Except when oxygen consumption was being measured, the swim chamber was flushed continuously to ensure the water was fully aerated. Oxygen consumption, either at rest or during swimming, was measured by turning the flush pump off, thereby sealing the swim chamber. Oxygen tension then decreased as the fish consumed the oxygen. Oxygen tension was allowed to decrease until saturation reached ~90 %, a period of about 10min, before the flush pump restarted, restoring the oxygen tension of the water in the swim chamber back to full air saturation, again after a period of about 10min. Oxygen consumption was calculated from the rate of decline in oxygen tension, the volume of the swim chamber and the solubility of oxygen in seawater at the experimental temperature (cf. (Lee et al., 2003; Schurmann and Steffensen, 1997)). Values for M_{O_2} in mg O_2 kg^{-1} h^{-1} were therefore recorded every 10 to 25 min depending on the duration of the flush cycles and represent the integrated oxygen consumption over a single measurement phase. M_{O_2} values were then converted from milligrams of O_2 per kilogram per hour to micromoles of O_2 per kilogram per minute before further processing. During swim trials, between one and

three measurements of M_{O_2} (i.e. over several flush cycles) were recorded at each water speed. Mean DBA (as both ODBA and VeDBA, see Eqs. 2 & 3) values were calculated from the raw logger data for the corresponding oxygen consumption measurement periods.

Once acclimated to the swim chamber, swim trials were conducted during daylight hours between 09:00 and 17:00 h each day, to reduce any diurnal effects on metabolism (Lefrançois et al., 2001; Page et al., 2011). During trials, oxygen consumption was measured at a range of speeds up to the point where the fish was unable to hold station. Starting from rest, water speed was increased in steps of 0.125 BL s^{-1} (bass and trout) or 0.2 BL s^{-1} (eel). If a fish was unable to maintain steady swimming performance during a trial, the water speed was decreased briefly to enable the fish to regain position, before a further attempt at the failed speed was initiated. To ensure that oxygen consumption values were representative of set swimming speeds, data were only used from periods when the fish was holding station and swimming steadily (Gleiss et al., 2010; Grøttum and Sigholt, 1998; Ohlberger et al., 2007).

The presence of the fish within the swim chamber can cause a 'solid blocking effect', whereby the water speed increases as it flows past the fish (Bell and Terhune, 1970). If the cross-sectional area of the fish exceeded 10 % of the cross-sectional area of the chamber, water speeds were corrected to account for this effect.

At the end of the experiments for eel, tags were removed by cutting the sutures at each corner before fish were released back into a tank with conspecifics. For bass and trout experiments, fish were humanely killed. Subsequently, but prior to its removal, the logger was calibrated *in situ* by slowly rotating the fish through 360 ° in each of the 3 axes of acceleration. This allowed correction of the acceleration values where the logger was not aligned precisely with respect to the vertical. The final length, weight and sex of the fish were then measured, and the gills were checked for ecto-parasites which may have affected oxygen consumption (Powell et al., 2005). No ecto-parasites were found on any of the fish, so all results were used in the following data analysis.

Data Analysis

Oxygen consumption

M_{O_2} was calculated from raw oxygen consumption values to account for size differences between test individuals (Clarke and Johnston, 1999; Fonds et al., 1992; Sloman et al., 2006) such that:

$$M_{O_2} = V \cdot \left(\frac{\delta\, pO_2}{\delta\, t}\right) \cdot \propto M^{-1} \quad (1)$$

where V is the volume of the swim tunnel, $\propto$ is the oxygen solubility, pO_2 is the partial pressure of oxygen, t is time and M is the wet weight of the fish. M_{O_2}values were standardised to 800g using an allometric scaling exponent of 0.8 which has been used for a number of other fish species (Bushnell et al., 1984; Edwards et al., 1972; Fry, 1971).

Dynamic Body Acceleration

Dynamic body acceleration (DBA) can be used to obtain estimates of activity level, using either ODBA or VeDBA. To identify the most appropriate DBA metric for tracking activity-specific energy of fish, both ODBA and VeDBA were derived from tri-axial acceleration of sea bass. Each metric was regressed against oxygen consumption to identify the DBA metric with the strongest correlation. Sea bass datasets were used for this initial comparison, with the optimal metric applied to trout and eel acceleration datasets.

ODBA is calculated by summing the dynamic acceleration values of the fish, and has been shown to be a marginally better proxy for oxygen consumption for a range of species including humans (Qasem et al., 2012). However, in situations where the orientation of the device is not necessarily aligned precisely with respect to the vertical, VeDBA (the vectorial sum of dynamic acceleration values) may be a better proxy (Qasem et al., 2012); though no studies to date have compared ODBA with VeDBA for fish with accelerometers implanted intraperitoneally.

ODBA and VeDBA were calculated by first removing the static component of acceleration (the acceleration due to gravity) from the acceleration time series

using the method detailed by Shepard et al., (2008). Briefly, this involved identifying the optimal smoothing algorithm for a steadily swimming fish. Smoothing intervals are species-specific (with the gait and tail beat frequency influencing the optimal smoothing algorithm). After testing a range of smoothing parameters, 2 s was appropriate for separating the static from the dynamic acceleration for all species in this study. Data were then converted to absolute values to reflect the change in acceleration from 0 (irrespective of the values being negative or positive) before the vectorial sum (VeDBA) or the sum (ODBA) was calculated using the following equations:

$$VeDBA = \sqrt{{A_x}^2 + {A_y}^2 + {A_z}^2} \quad (2)$$

$$ODBA = A_x + A_y + A_z \quad (3)$$

where A_x, A_y and A_z are the absolute dynamic acceleration values measured with respect to the X, Y and Z axes, respectively (cf. Qasem et al., 2012). Mean VeDBA and ODBA were calculated every 2s to incorporate the peak and trough of the fish tail beat prior to further processing. The measuring period for each M_{O_2} value was used to extract the mean VeDBA or ODBA value over the corresponding time.

It has been noted for some species (e.g. European eel, *Anguilla anguilla*; (Methling et al., 2011)) that tagging can affect the cost of transport (COT), a measure of the relative cost of motion at each speed, that can be used to estimate optimal swimming speed, i.e. the speed at which COT is a minimum (Schmidt-Nielsen, 1972). To assess whether there was a difference in COT before and after logger implantation, the minimum cost of transport (COT_{min}) and optimal swimming speed (U_{opt}) were calculated and compared as a function of the ambient temperature, such that:

$$COT = \left(\frac{a}{U}\right) + b \cdot U^{(c-1)} \quad (4)$$

where a, b and c are all constants and U is the speed of the fish in body lengths per second. From the COT relationship U_{opt} and the corresponding oxygen consumption at this speed, COT_{min} values were also determined.

The relationship between M_{O_2} and water speed and between M_{O_2} and acceleration was identified using a linear mixed model, with temperature, total length and sex as covariables and with the fish's identification number (ID) as a random factor. Sex was not included as a covariables for trout and eel experiments, as all fish were female. Models were compared and selected based on values of Akaike's information criterion (AIC) (Sakamoto et al., 1986). The standard metabolic rate (SMR_U) was calculated by extrapolating the oxygen consumption value to zero water speed (Brett, 1965; Dewar and Graham, 1994; Schurmann and Steffensen, 1997). To calculate the SMR for regressions between DBA and oxygen consumption, first, the DBA at zero water speed (DBA_r) was calculated for experiment group, yielding the resting DBA level (which includes minor adjustments in posture at rest). This DBA_r value was then incorporated into the DBA/M_{O_2} regressions from the mixed effects model to calculate the SMR_{VeDBA} and SMR_{ODBA} values (for VeDBA and ODBA regressions, respectively).

These SMR values were then compared with previously published values for fish at different temperatures. SMRs were regressed against temperature using the following exponential relationship:

$$SMR = a \cdot e^{(b \cdot T)} \quad (5)$$

where a and b are both constants and T is the temperature in degrees Celsius.

Activity levels may be suppressed in the respirometer compared to the holding tank (Peake and Farrell, 2004), affecting the range of motion that we are able to attain in the study. To assess whether there was a difference in the activity level of fish in the holding tank and the respirometer, frequency distributions of DBA were compared by extracting the maximum and modal values of the frequency distributions in the 2 situations.

Statistics

Data were tested for normality using the Anderson Darling test for normality, and, if data were not normally distributed, the values were log-transformed to satisfy requirements for subsequent statistical tests. Where appropriate, values are shown as means (±SD), and the level of statistical significance was set at $\alpha = 0.05$.

Results

Overview of experiments and condition

We have simultaneously measured dynamic body acceleration (both as ODBA and VeDBA) and oxygen consumption at rest and while swimming at a range of water speeds in 9 European sea bass, *Dicentrarchus labrax*, 6 rainbow trout, *Oncorhynchus mykiss*, and 9 European eel, *Anguilla anguilla*.

Experiments with sea bass were carried out at ambient temperatures between 5.5 and 17.5 °C (Table 4.1). However, during the 5 to 6 month experiments, 3 fish (Fish 4, 5 and 6) expelled their loggers, resulting in no data for summer temperatures (13 and 14 °C) for these individuals. Flume tests after respirometry trials showed no detectable background bacterial respiration in the swim chamber; thus, no correction of oxygen consumption values was needed.

Metabolic rates and activity level

Oxygen consumption scaled exponentially with swimming speed at all temperatures (Fig.4.2). For statistical comparison of M_{O_2} with U, M_{O_2} values were log-transformed. Water speed had a significant positive effect on oxygen consumption for bass (Eqn 6), trout (Eqn 7) and eel (Eqn 8). In conjunction with water speed, oxygen consumption also significantly increased with temperature for bass:

$$Log(M_{O_2}) = 1.198(U) \cdot 0.003(T) + 2.483 \quad (6)$$

$$Log(M_{O_2}) = 0.946(U) + 2.864 \quad (7)$$

$$Log(M_{O_2}) = 1.008(U) + 3.260 \quad (8)$$

The relationship between dynamic body acceleration and oxygen consumption was developed using a mixed model for sea bass, where temperature, total length and sex were used as predictor variables and fish ID was a random factor. Acceleration (ODBA/VeDBA) and temperature were the only significant (P<0.05) predictor variables with positive effects on oxygen consumption (Table 4.2, Fig.4.2). Total length and sex were non-significant (P>0.05; Table 4.3):

$$M_{O_2} = 961.961(VeDBA) \cdot 2.7203(T) - 2.596 \quad (9)$$

$$M_{O_2} = 584.676(ODBA) \cdot 1.568(T) + 1.100 \quad (10)$$

Table 4.2 Significance of variables and variable interactions for water speed or dynamic body acceleration (*VeDBA/ODBA*) and oxygen consumption for nine European sea bass

Variables	*P* values		
	Bass	Trout	Eel
M_{O_2} & Water Speed			
Water Speed	<0.0001	<0.0001	<0.0001
Temperature	<0.0001	-	-
TL	0.0690	0.0658	0.070
Sex	0.6920	-	-
M_{O_2} & VeDBA			
VeDBA	<0.0001	<0.0001	<0.0001
Temperature	<0.0001	-	-
TL	0.0713	0.6867	0.4934
Sex	0.9098	-	-
M_{O_2} & ODBA			
ODBA	<0.0001	-	-
Temperature	<0.0001	-	-
TL	0.1003	-	-
Sex	0.9528	-	-

There was no significant difference between VeDBA and ODBA (P=0.995), though the AIC was lower for VeDBA (2181) in comparison to ODBA (2191).

VeDBA was therefore used as the dynamic body acceleration metric for further comparisons involving trout and eel. Mirroring results for bass, there was a significant, positive increase of oxygen consumption with VeDBA for both trout (Eqn 11) and eel (Eqn 12), with no significant effect of total length (Table 4.3):

$$M_{O_2} = 1145.618(VeDBA) + 1.580 \quad (11)$$

$$M_{O_2} = 359.165(VeDBA) + 31.917 \quad (12)$$

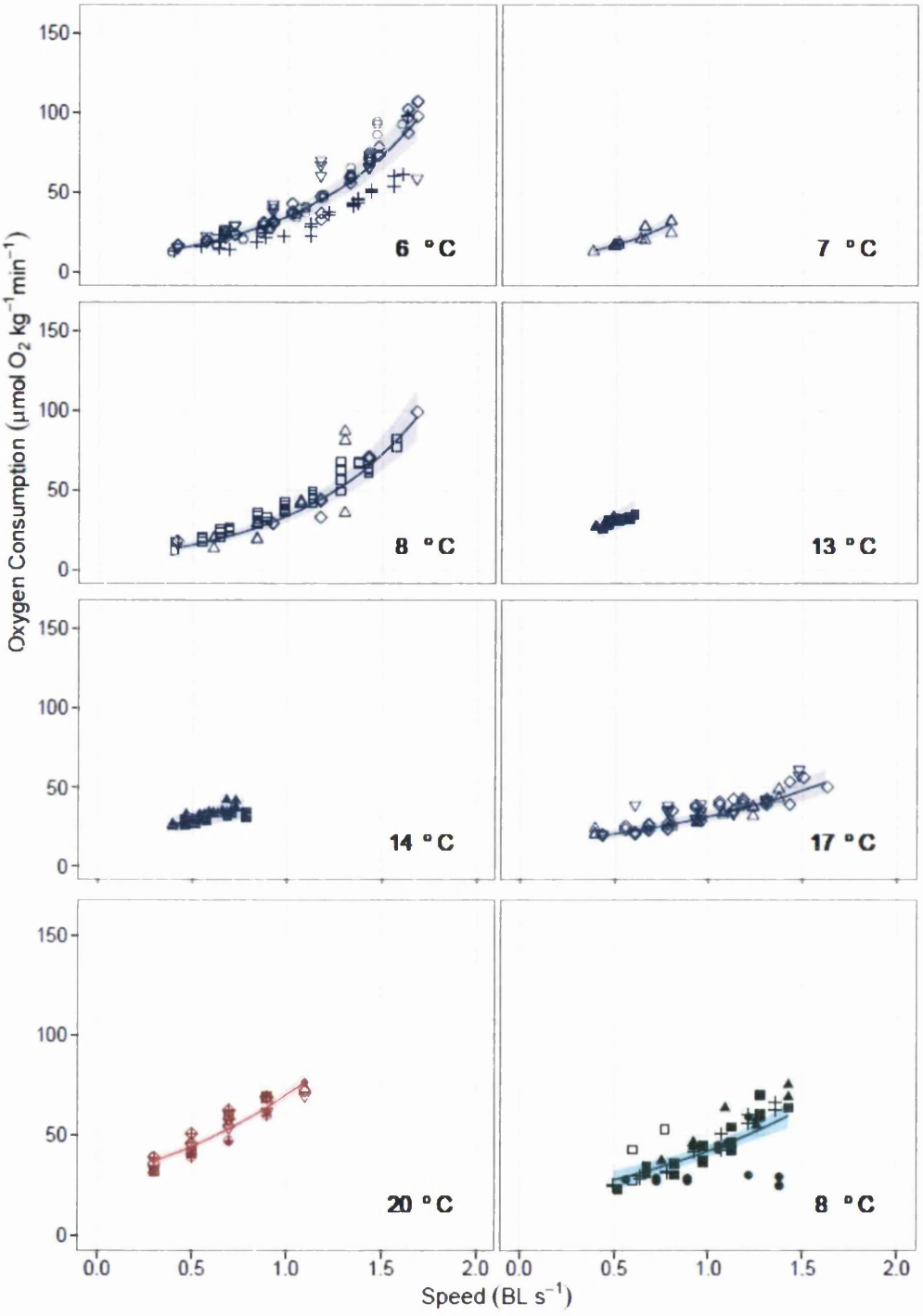

Figure 4.2. Oxygen consumption as a function of water speed for European sea bass, *Dicentrarchus labrax* (blue), European eel, *Anguilla anguilla* (red) and rainbow trout, *Oncorhynchus mykiss* (green). The temperature is denoted at the bottom right of the plot (individuals are represented by different symbols). Data was fitted with a linear mixed effect model after oxygen consumption was log transformed, therefore each line represents the relationship between M_{O_2} and water speed at a given temperature. Note the low spread of values at 7, 13 & 14°C, which results from unsteady swimming of the sea bass at relatively low speeds (0.7 – 0.8 BL s^{-1}).

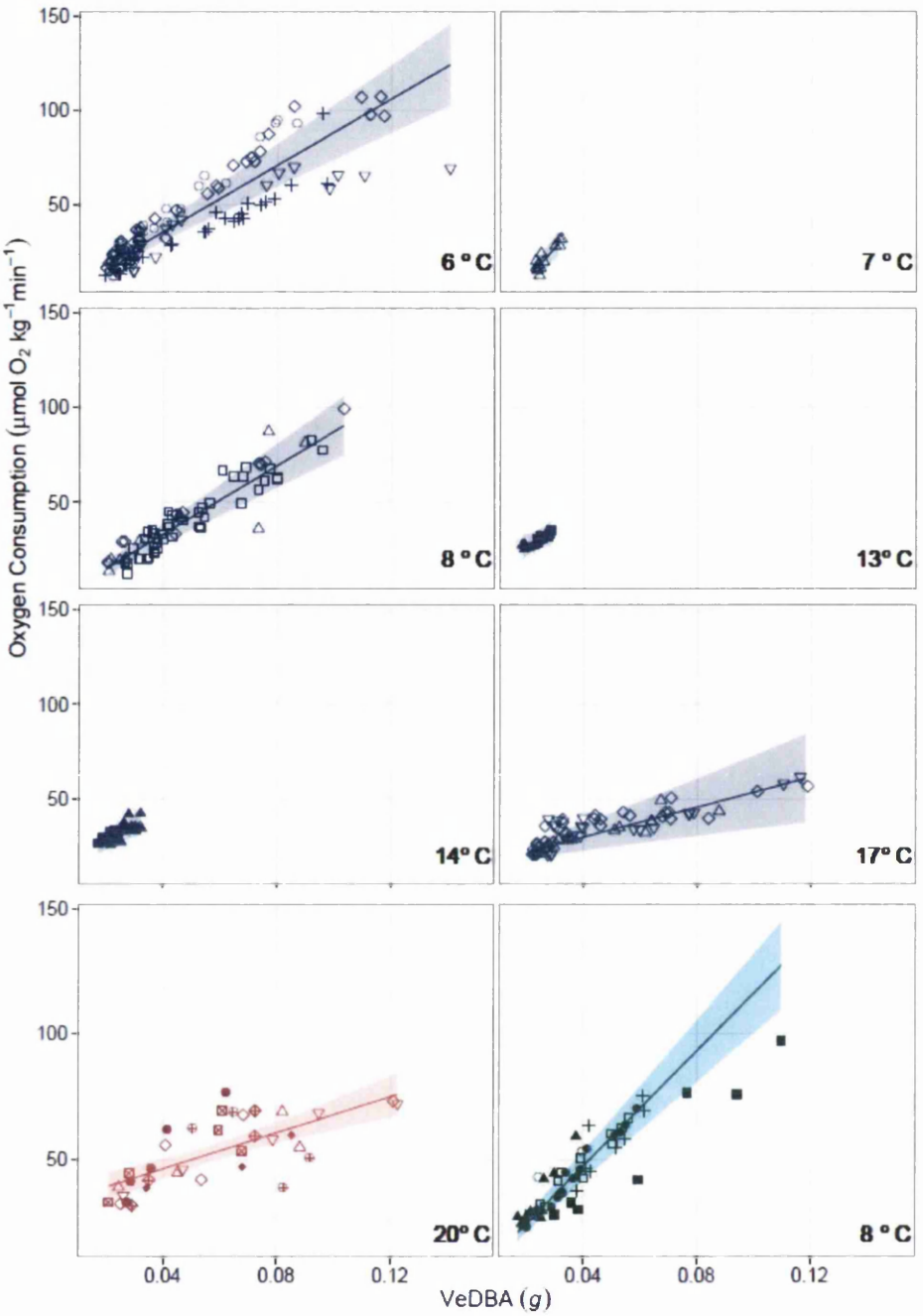

Figure 4.3. Oxygen consumption as a function of Vectorial Dynamic Body Acceleration (*VeDBA*) for European sea bass, *Dicentrarchus labrax* (blue), European eel, *Anguilla anguilla* (red) and rainbow trout, *Oncorhynchus mykiss* (green). The temperature is denoted at the bottom right of the plot (individuals are represented by different symbols). Data was fitted with a linear mixed effect model, therefore each line represents the relationship between M_{O_2} and *VeDBA* at a given temperature. Note the low spread of values at 7, 13 & 14 °C, which results from unsteady swimming of the sea bass at relatively low activity levels (0.04*g*).

There was an exponential increase in VeDBA with water speed (U, BL s^{-1}), for bass $VeDBA = 0.0109 \cdot e^{(1.3911 \cdot U)}$, ($P$<0.001, r^2=0.87), trout $VeDBA = 0.0171 \cdot e^{(0.7353 \cdot U)}$, ($P$<0.001, r^2=0.40) and eel $VeDBA = 0.0255 \cdot e^{(1.1678 \cdot U)}$, ($P$<0.001, r^2=0.45) (Fig.4.4).

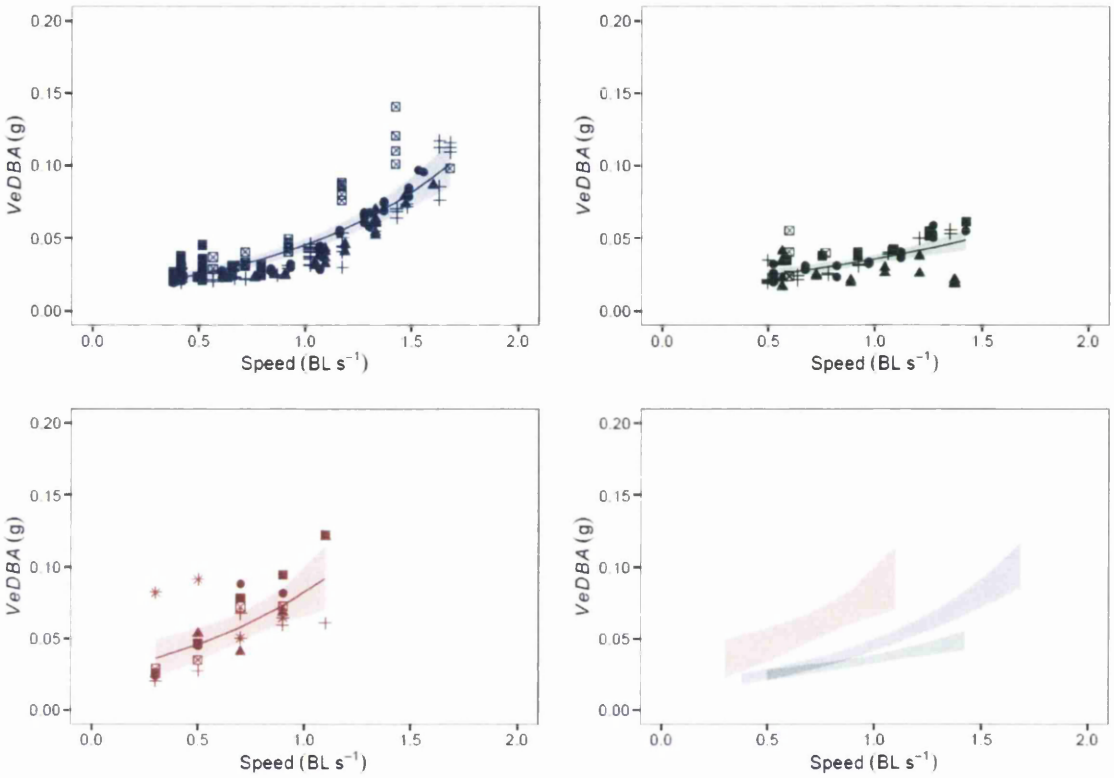

Figure 4.4. Vectorial Dynamic Body Acceleration (*VeDBA*) as a function of water speed (BL s^{-1}) for nine European sea bass, *Dicentrarchus labrax* (blue), six rainbow trout, *Oncorhynchus mykiss* (green) and nine European eel, *Anguilla Anguilla* (red). The regression lines correspond to the exponential increase in *VeDBA* with water speed. Data was combined from all experiments in this comparison, as the fish speed and *VeDBA* are both independent of temperature.

Standard metabolic rate

Standard metabolic rate (SMR_U) was derived by extrapolating the oxygen consumption to zero swimming speed for sea bass. These SMRs were regressed against ambient temperature and plotted together with those cited in several previously published studies using Eq. (5). SMRs derived from this study were lower than those previously published at similar temperatures (Fig.4.6). Though there was

a strong correlation between SMR and temperature when all values were combined (Eq. 13) in contrast to when only the previously published values were used (Eq. 14):

$$SMR = 7.68 \pm 1.06 \cdot e^{(0.069 \pm 0.006 \cdot T)} \ (P<0.0001, r^2=0.82) \quad (13)$$

$$SMR = 13.83 \pm 2.56 \cdot e^{(0.047 \pm 0.008 \cdot T)} \ (P<0.001, r^2=0.80) \quad (14)$$

where T is the temperature (°C).

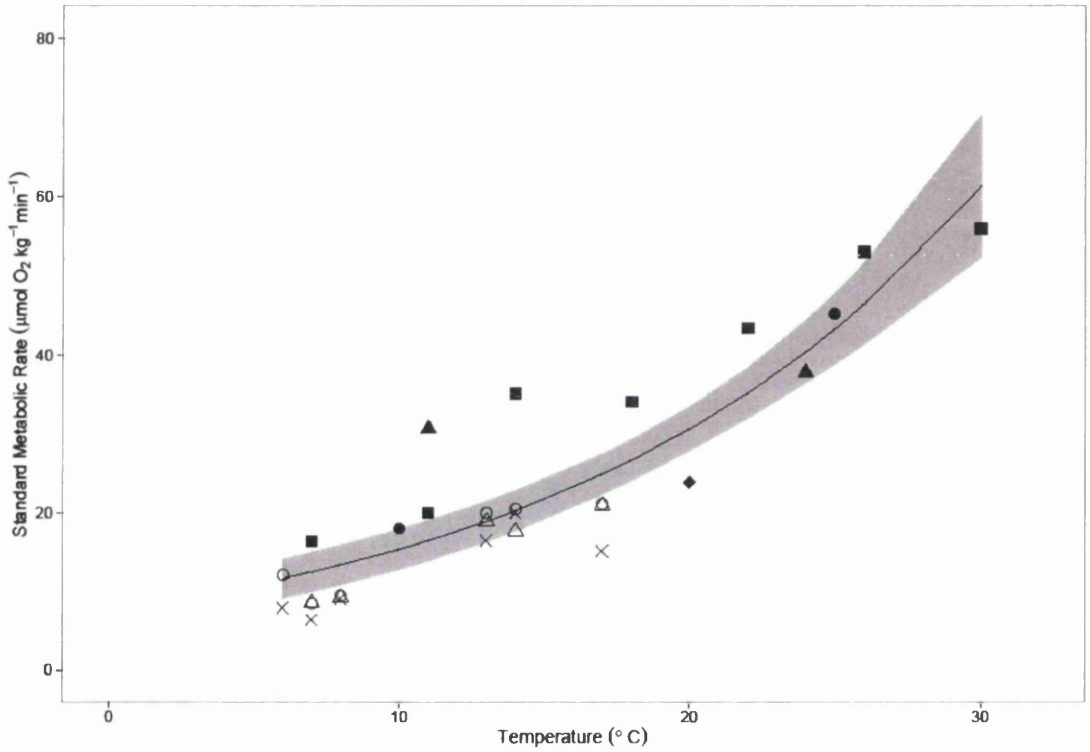

Figure 4.5. Standard metabolic rate (*SMR*) as a function of water temperature for European sea bass (*Dicentrarchus labrax*). *SMR* values derived from models between oxygen consumption and *VeDBA* (○), *ODBA* (△) and swimming speed (×) together with previously published values: ●: Claireaux et al. (1999), ■: Claireaux et al. (2006), ◆: Herskin & Steffensen (1998), ▲: Surea (1995). The shaded area corresponds to the 95 % confidence limits of the exponential increase in SMR with temperature when all data was combined (Eqn 13: $SMR = 7.68 \pm 1.06 \cdot e^{(0.069 \pm 0.006 \cdot T)}$, ($P<0.0001$, $r^2=0.82$)).

Cost of transport before and after logger implantation

Cost of transport was compared for sea bass both before and after tag attachment in relation to water temperature. COT_{min} values were higher before than after tagging (Fig.4.6). For example, at 8 °C COT_{min} ranged from 74.5–102.4 μmol

O_2 kg^{-1} BL^{-1} before tagging to 62.7–77.7µmol O_2 kg^{-1} BL^{-1} after tagging. There was a significant linear relationship between temperature and COT_{min} in post-tagging trials (r^2=0.79, P<0.01; Fig.4.6). Despite this relationship between COT_{min} and temperature, there was no significant relationship between U_{opt} and temperature (r^2=0.51, P=0.11).

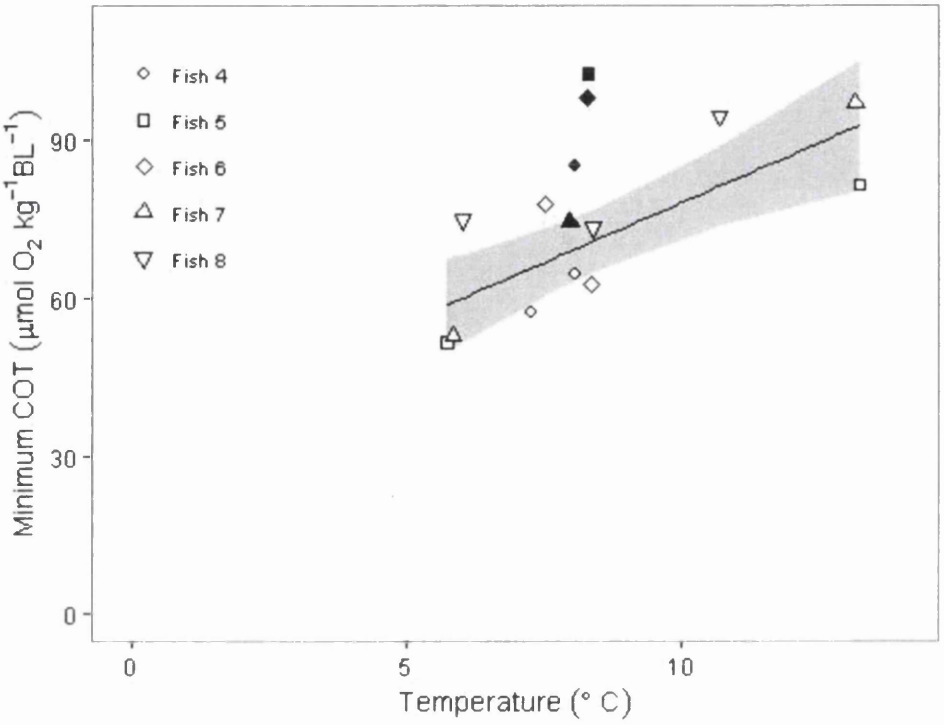

Figure 4.6. Minimum cost of transport (COT_{min}) as a function of ambient temperature for individual European sea bass (*Dicentrarchus labrax*) prior to logger implantation (black symbols) and after (white symbols). Individuals are represented by symbols. The solid line corresponds to the linear regression between temperature and COT_{min} after tagging (Eqn 11: $COT_{min} = 32.72 + T \cdot 4.52$, r^2=0.79, P=0.004). The shaded area represents the 95 % confidence limits of the regression line.

DBA in the respirometer and holding tank

To assess whether there was a difference in the activity level of fish in the holding tank and in the swim chamber, frequency distributions of VeDBA in these 2 situations were compared (Fig.4.7). Activity levels (VeDBA) were consistently higher in the holding tank compared to the swim chamber, with higher modes and maximum levels (Table 4.3). Whilst in the swim chamber, fish swam progressively faster up to the maximum sustained speed; as the water speed increased, the

spectrum of VeDBA values also increased encompassing the range of activity in the holding tank (Fig.4.7c).

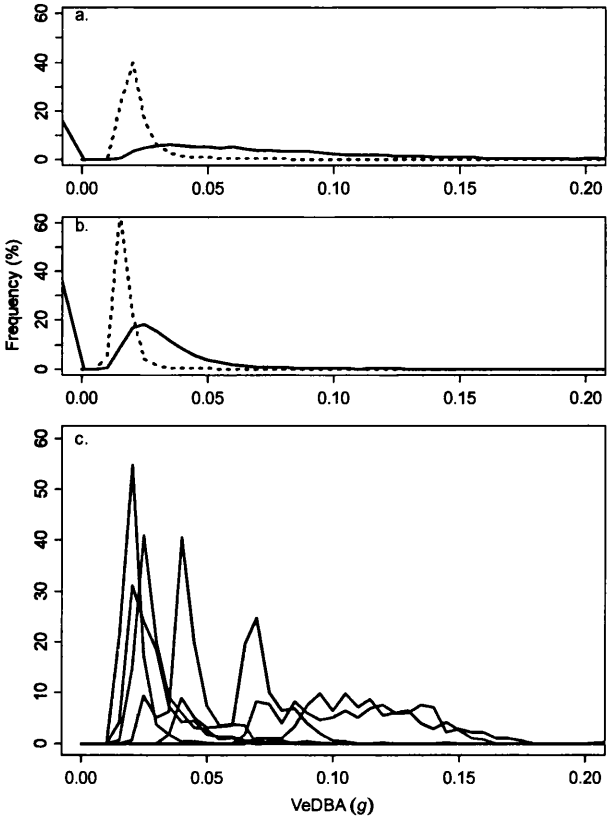

Figure 4.7. Frequency distribution of *VeDBA* (g) whilst in the swim chamber (dotted line) and holding tank (solid line) for European sea bass 1 (a.) and 2 (b.). Frequency distribution of *VeDBA* whilst swimming at a range of set speeds for fish 2 in the respirometer (from 0.3 to 1.6 BL s^{-1}, red to green respectively).

Table 4.3. Statistics from frequency distributions of *VeDBA* for European sea bass (*Dicentrarchus labrax*) in a swim chamber and holding tank

		Location	
Statistic	Fish	Swim chamber	Holding tank
Mode	1	0.0186	0.0349
	2	0.0261	0.0523
Max	1	0.0854	0.0935
	2	0.663	1.1590

Discussion

Our results indicate that there is a significant correlation between dynamic body acceleration (measured here as ODBA and VeDBA) and activity-specific energy expenditure for all fish species in this study. Both oxygen consumption and dynamic body acceleration increased with water speed at all temperatures. The exponential relationship between swim speed and oxygen consumption is broadly similar to those previously reported for other teleost fish (Farrell, 2007; Melzner et al., 2009) including European sea bass (Claireaux and Lagarde, 1999; Claireaux et al., 2006), European eel (Methling et al., 2011) and rainbow trout (Alsop and Wood, 1997). Our general finding that DBA scales linearly with oxygen consumption also confirms previous results from both terrestrial and aquatic species (Gleiss et al., 2009; Halsey and White, 2010; Wilson et al., 2013).

Effects of tagging and cost of transport

Recent studies have shown that fish carrying externally attached devices incur increases in the energetic cost of swimming at optimal speeds (Methling et al., 2011). For experiments with sea bass and rainbow trout, data loggers were implanted internally into the peritoneum, to reduce the effect of logger drag. Due to space limitations, tags had to be attached externally to European eel, potentially affecting energy use, though for the scope of this study, the effect of external logger attachment was not assessed.

As a model study, we compared the cost of transport for the steadily swimming sea bass before and after tagging, to establish whether internal implantation influenced swimming performance and energy use. Our results indicate that the COT_{min} was consistently lower after tagging. The unexpected higher COT prior to tagging may reflect increased oxygen consumption due to stress induced by the new and unfamiliar surroundings in the swim chamber (Martins et al., 2011). This analysis therefore does not fully establish that the fish were unaffected by tagging, but rather suggests that the fish became accustomed to the experimental apparatus over time and were therefore less stressed in post-tagging trials than in pre-tagged trials, with a consequent reduction in oxygen consumption. However,

results do indicate that tagging per se did not lead to any increase in the energetic costs of swimming for bass. Post tagging COT_{min} also increased exponentially with temperature, a relationship also reported for other fish species, including chub mackerel *Scomber japonicus* (Dickson et al., 2002). Therefore, we conclude that, for sea bass at least, it is difficult to assess the effect of tagging using pre- and post-tagging trials on the same individuals because stress, and therefore oxygen consumption, may be reduced over time as the fish become accustomed to the experimental apparatus, despite our efforts to acclimatise the bass in this study to being handled by presenting them with 'net challenges' each day.

Differences between VeDBA and ODBA

Dynamic body acceleration can be calculated by either deriving the sum (ODBA) or the vectorial sum (VeDBA) of the acceleration. ODBA has previously been shown to be a better proxy for oxygen consumption in a range of species, including humans (Qasem et al., 2012). However, as the accelerometer is not necessarily aligned precisely with respect to the vertical in the current application, VeDBA may be a better proxy. To identify the metric suitable for studies using similar methods, results from bass were compared. The accelerometer was implanted in the peritoneal cavity of the bass, standardising the position of the device between individuals after the initial recovery period (during which time the logger becomes encapsulated in connective tissue). We have therefore compared the fits of both VeDBA and ODBA with oxygen consumption to assess which DBA metric provided the best fit. There was no significant difference between the mixed models for ODBA or VeDBA, mirroring results from a recent study (Qasem et al., 2012), although the model with VeDBA had a lower AIC than ODBA; suggesting that VeDBA is a better proxy for oxygen consumption than ODBA. This finding is similar to those of Qasem et al., (2012) who predicted that this should be the case in situations where a consistent orientation of the data logger cannot be guaranteed between individuals.

Standard metabolic rate

Our values of SMR (i.e. the oxygen consumption when the fish is at rest) derived by extracting the oxygen consumption at zero swimming speed (SMR_U) or minimum activity level (SMR_{VeDBA} and SMR_{ODBA}) are somewhat lower than those reported previously for sea bass (Claireaux and Lagarde, 1999; Claireaux et al., 2006; Herskin and Steffensen, 1998) (Fig.4.4). Though, there is a strong correlation (r^2) between SMR and temperature when all results are combined (Eq. 7).

In the present study we estimated the SMR of bass (800g) at 13°C to be between 16.45 and 19.89μmol kg^{-1} min^{-1} as derived from water speed and VeDBA regressions, respectively. After correcting for mass-specific differences, the equivalent SMR calculated from previous studies of sea bass would be 25.48 μmol kg^{-1} min^{-1} (Eq. 8). Differences may be due to the higher mean temperature in the other studies (18.17 ± 7.46 °C), in contrast to the present study (10.83 ± 4.45 °C). Other factors which may have resulted in the observed differences include handling stress or population differences of genetically distinct stocks. Although all fish in both the present and cited studies were obtained from hatcheries (Claireaux and Lagarde, 1999; Claireaux et al., 2006; Herskin and Steffensen, 1998), genetic differences between populations may affect metabolic rates (Gamperl et al., 2009). Alternatively, the differences may simply reflect errors in deriving SMR by extrapolating from regressions where the closest data points are still some way from the intercept.

This is particularly germane in studies seeking to describe SMR rather than resting metabolic rate (RMR) (Carlson et al., 2004). RMR is the metabolic rate of an animal at rest, whilst the SMR is the minimum metabolic rate required to sustain life. In contrast to the RMR, the SMR can be extrapolated from an active animal, and therefore can be over/underestimated if there are inaccuracies in the regression slope of the active animal.

The problem with swim tunnels

Swim tunnel studies have frequently been used to shed light on relationships between swimming speed (or performance) and metabolism in fish (Claireaux and

Lagarde, 1999; Gleiss et al., 2010; Steinhausen et al., 2005). However, many authors question the validity of the capacity of swim tunnel work to replicate natural swimming conditions (Nelson et al., 2002). Novel environments may cause the fish to behave abnormally for prolonged periods, resulting in an increased oxygen uptake (Bonga, 1997), and issues may arise due to the size or design of the swim tunnel (Tang and Boisclair, 1993), with animal movement potentially limited at crucial times such as during gait transition (Peake and Farrell, 2004). In the present study, the limited space in the swim chamber compared to the holding tank had an immediately quantifiable effect on the activity level (DBA profile) of the bass. Nonetheless, the range of activity levels achieved during the swim trials (water speeds of between 0.3 and 1.6 BL s^{-1}) successfully spanned the range of activity exhibited by the fish in the holding tank. Though, it must be noted that, the forced steady swimming used during respirometry trials is not always representative of the behaviour that fish exhibit in unimpeded (free-swimming) scenarios, and may result in erroneous oxygen consumption measures.

Loggers versus transmitters

Loggers and transmitters can be used to record the acceleration of animals both in the field and laboratory. Acceleration in this study was recorded using a logger, which provides continuously sampled acceleration at a fine resolution. Other recent studies have shown the value of using acceleration transmitters to track fish (Wilson et al., 2013), which combine and transmit acceleration every few seconds.

There are both benefits and caveats when using acoustically transmitted and logged acceleration. Both acoustically transmitted and logged acceleration provide an 'overall' activity level (DBA) indicator, which can be used to calculate activity-specific energy use (Gleiss et al., 2010; Wilson et al., 2013). However, logged acceleration can also be used to extract more detailed information about the behaviour of the animal (Broell et al., 2013; Brownscombe et al., 2013; Whitney et al., 2008; Whitney et al., 2010), for example as a means to identify specific behavioural events including feeding (Broell et al., 2013; Gleiss et al., 2013). We are unable to extract these behaviours using transmitters, though loggers are not always practical or feasible to use as they must be retrieved at the end of the study.

Researchers therefore need to select the appropriate recording or transmitting device de pending on their research aims and the likelihood that devices will be easily retrieved at the end of the study.

Conclusion

Electronic data loggers (data storage tags) have previously been shown to provide unparalleled insights into the vertical movement and habitat selection of aquatic animals in the wild (Hays et al., 2008; Metcalfe et al., 2005; Righton et al., 2010; Sims et al., 2006). Now, with the incorporation of tri-axial accelerometers, these devices promise to provide a repeatable and robust method for estimating activity-specific metabolic rates. The correlations between DBA and M_{O_2} demonstrate that DBA can be used as a metric to estimate activity-specific metabolic rates in fish (Gleiss et al., 2010; Wilson et al., 2006; Wilson et al., 2013), and reconfirms the use of acceleration as a proxy for activity-specific metabolic rates in the field (Halsey et al., 2011). Acceleration data loggers can be implanted internally into fish and can, with an appropriate duty cycle, have a long-operating life. This suggests that DBA could be a useful proxy for estimating activity-specific energy expenditure from fish in mesocosm or field studies over extended periods where other methods (e.g. oxygen consumption) would be more problematic, although details of the physiological inter-relationships between temperature, specific dynamic action, basal, standard and active metabolic rates would need to be derived from laboratory studies before DBA could be used to provide a complete estimate for field metabolic rate. Nonetheless, DBA would appear to be a valuable tool to gain a clearer picture of how fish make energy-based tradeoffs between different levels of activity when faced with confliction or competing demands arising from increased and combined environmental stressors.

An additional potential application of accelerometers in relation to activity-specific energy expenditure is the capability of this technology to record very short periods of intense activity, which, for most fish, is supported by anaerobic metabolism. White muscle (the bulk of the muscle in most fish) has a poor blood supply, and white muscle activity relies largely on anaerobic metabolism. White muscle, therefore, functions largely as an 'emergency power pack' during burst

swimming that is often essential for predator avoidance or prey capture; behaviours that, particularly in the case of predator avoidance, can be critical to survival. However, most laboratory-based studies on metabolic processes in fish can only be carried out when the animal is in a steady-state, either resting or swimming, and metabolising aerobically. This is because the experimental methods used are unable to capture sufficient physiological information during brief episodes of intense activity. Consequently, it has previously been difficult to investigate how such life-critical anaerobic capabilities are affected by temperature and other stressors (e.g. hypoxia, acidification, starvation). Accelerometry, therefore, offers, for the first time, the potential to investigate the effects of environmental temperature and other environmental stressors (alone and in combination) on both the aerobic and anaerobic capabilities of fish. Furthermore, this technology can be readily applied in both laboratory (for ground-truthing) and field situations; therefore offering a unique capability for understanding how environmental stressors affect fish energetics, particularly their life-critical, anaerobic capacities, in their natural environment and how they make tradeoffs between different activities that allow them to cope with environmental stress.

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Chapter 5

Estimating Activity-Specific Energy Expenditure in teleost fish, using accelerometer loggers: *Anaerobic Activity*

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S.R. Wright was responsible for data collection, analysis and writing the manuscript. All authors proof read and made amendments to the manuscript.

Abstract

The anaerobic component of energy use is difficult to quantify in free-swimming fish, yet can have implications for energy budgets and survival. As a means to derive anaerobic energy use, excess post-exercise oxygen consumption (EPOC) was estimated at a number of activity levels for European sea bass, *Dicentrarchus labrax* (n=9) tagged with tri-axial accelerometer data loggers. The activity level of fish was calculated as the vectorial dynamic body acceleration (VeDBA) whilst fish swam in a swim-tunnel respirometer at temperatures between 5 °C and 18 °C. EPOC was compared to exercise duration, intensity and the amount of time required for oxygen consumption to return to aerobic limits to assess whether anaerobic energy use can be estimated solely from the acceleration of fish. EPOC reached a maximum of 912 μmol O_2 and significantly increased with exercise intensity and exercise duration, with no significant effect of temperature. The amount of time required for oxygen consumption to return to aerobic limits significantly increased with EPOC, with a maximum of 70 minutes. The results show that, with validation experiments, accelerometers can be used to estimate exercise intensity (VeDBA) and duration, which can then be used to predict EPOC and thus recovery time from high intensity, anaerobic activities of fish in the laboratory and field.

Introduction

Numerous studies have assessed the cost of steady-state locomotion in fish (Claireaux et al., 2006; Clark et al., 2010; Dickson et al., 2012; Wright et al., 2014), though the dynamic nature of fish behaviour results in a complex interaction between both fully aerobic steady-state swimming and anaerobic burst swimming. The relative importance of aerobic and anaerobic locomotion for different fish species is reflected in differences in ratios of red to white muscle fibres (Drazen et al., 2013; Greek-Walker and Pull, 1975). Species with a higher ratio of red to white muscle have an increased aptitude for aerobic locomotion and include long distance migrators, such as Atlantic salmon, *Salmo salar*. A lower ratio of red to white muscle indicates species better equipped for anaerobic locomotion, such as "fast-starts", which can lead to higher than aerobically deliverable energy use. However, for many species these fast-starts provide an effective and efficient means to catch prey (Harper and Blake, 1991; Webb and Skadsen, 1980), even at a higher energetic cost than using aerobic modes of locomotion. The increase in post-exercise oxygen consumption (M_{O_2}) required for anaerobic locomotion was previously defined as oxygen debt (Hill et al., 1924), but is now more accurately defined as excess post-exercise oxygen consumption (EPOC) and corresponds to the elevated metabolism occurring after vigorous exercise (Gaesser and Brooks, 1984). This elevation in metabolism has implications for the energy budgets of fish, as energy available for other functions can be limited during repayment.

EPOC has two phases, a rapid phase and prolonged phase (Gaesser and Brooks, 1984). The rapid phase subsides relatively quickly (within ~ 1 h), and involves metabolic processes believed to be responsible for a number of physiological changes including the replenishment of O_2 stores in blood and muscle, the resynthesis of ATP and creatine phosphate, and the removal of lactate (Børsheim and Bahr, 2003). The prolonged phase decays mono-exponentially with a half-life in the order of several hours. The reason for the prolonged phase is yet to be fully understood, though it may be attributed, at least in part, to the triglyceride/ fatty acid cycle and a shift from the use of carbohydrate to fat as a substrate source (Børsheim and Bahr, 2003). Previous studies note that this prolonged phase can be absent in humans when exercise bouts are at intensities corresponding to less than 50% of the

maximal oxygen use (Bahr et al., 1992), highlighting the importance of tracking the intensity of exercise prior to EPOC calculation. Thus, the method to induce EPOC can have a significant effect on the amount and duration of EPOC (Reidy et al., 1995). For example, for teleost fish, commonly used methods to induce EPOC include a relatively slow increase in water speed up to the maximum that the fish is able to swim (U_{crit}) (Lee et al., 2003; Svendsen et al., 2010; Zhao et al., 2012), or high intensity chasing of fish until exhaustion (Peake and Farrell, 2004; Scarabello et al., 1992). Both of these methods aim to attain the upper limit of anaerobic energy use of the fish. However, the performance of fish in the confines of a respirometer may be compromised (Nelson et al., 2002), with increases in oxygen use due to the novel environments (Bonga, 1997) rather than anaerobic energy use from locomotion (as explored further in Chapter 4, "The problem with swim tunnels"). Additionally, the maximal energy use attained using these methods does not fully reflect the behaviour of fish in the field, as fish activity can vary from stationary to high intensity. Thus, the issue of quantifying how EPOC is affected by the intensity and duration of exercise has not been assessed, though we may predict that both intensity and duration of locomotion will affect EPOC and concurrently energy use, as is apparent for other ectotherms including reptiles (Hancock and Gleeson, 2002). Other factors which may also affect EPOC include water oxygen level and temperature, as noted in experiments with salmon (Brett, 1964; Lee et al., 2003) and catfish (Zeng et al., 2010).

In addition to the direct energetic cost of EPOC, the amount of time required for animals to return to aerobic limits and to replenish energy stores has implications for their survival (Milligan, 1996), with the amount of energy available for other non-essential energy use, including locomotion, potentially compromised during this time. Differences in recovery time are suggested to reflect differences in the life stages of the test species (Lee et al., 2003). Though, other factors may also affect the recovery time including the exercise duration and intensity (Hancock and Gleeson, 2002). For example, studies with mice show that activities of short duration do not result in the same energetic cost per unit distance as activities of longer duration (Baker and Gleeson, 1998).

In the field of fish physiology the methods used to induce EPOC and to track their anaerobic energy use have been limited to closed experiments within the

laboratory (Baker and Gleeson, 1998; Lee et al., 2003). These studies provide a means to test how EPOC is affected by important environmental variables such as temperature (Zeng et al., 2010) and oxygen content of the water (Burleson et al., 1998; Svendsen et al., 2012), however, there are limitations to the kind of experiment that can be carried out within the laboratory, thus new methods are required to increase the capacity of researchers to track the anaerobic energy use of fish in the field.

Accelerometers provide a means of measuring the aerobic energy use of fish in the laboratory and the field (Gleiss et al., 2010; Wilson et al., 2013; Wright et al., 2014), but may also provide a tool to estimate anaerobic energy use. The present study uses European sea bass, *Dicentrarchus labrax*, as a model species to assess whether accelerometers may be used to estimate anaerobic energy use and the amount of time required for fish to return to aerobic limits.

Materials & Methods

1. Fish and Experimental Design

All equipment and tagging procedures are described in chapter 4. Briefly, nine wild caught European sea bass (*Dicentrarchus labrax*) were obtained from the Sea Life Centre, Great Yarmouth. Fish were transferred to the Cefas Laboratory, Lowestoft where they were placed in a holding tank at ambient water temperatures between 5.2 and 17.8 °C. Total fish lengths ranged from 45.1 to 55.0 cm, with total weights from 1100 g to 2200 g.

2. Accelerometers & Surgical Procedures

Acceleration was recorded using a G6a data storage tag (Cefas Technology; dimensions: 40 mm × 28 mm × 16.3 mm, 18.5 g in air, 6.7 g in seawater). Tags were set to record tri-axial acceleration (± 2 *g*) at 20 Hz. For a detailed description of the surgical attachment methods, see chapter 4. Briefly, 2- phenoxyethanol was used to anaesthetise fish prior to surgery; tags were then implanted within the peritoneal cavity where they were anchored in place using two sutures. Fish were left to recover

for a minimum of 4 weeks before respirometer experiments. The relatively long recovery time was used to ensure that the physiology and behaviour of the fish was not comprised during the respirometer trials.

3. Respirometry

Animal metabolic rate was measured in a Brett-type swim tunnel respirometer (Brett, 1965), using intermittent flow respirometry (Loligo Systems ApS). See chapter 4 for full details about the methods for recording oxygen consumption in the respirometer and experimental design. Fish were given a minimum of 17 hours to recover in the respirometer prior to swim trials. During this acclimation phase, the water speed was held constant at between 0.3 and 0.4 BL s^{-1}. Each swim trial was broken down into 'measurement', 'flush' and 'wait' phases. The measurement phase recorded the oxygen tension of the water in the swim chamber, with a M_{O_2} value every 10-25 minutes depending on flush, measure and wait durations. Swim trials were conducted during daylight hours to reduce any diurnal effects on metabolism (Page et al., 2011). Starting from rest, water speed was increased in steps of 0.125 BL s^{-1} (bass and trout) or 0.2 BL s^{-1} (eel). If a fish was unable to maintain steady swimming performance during a trial, the water speed was decreased briefly to enable the fish to regain position, before a further attempt at the failed speed was initiated, therefore the maximum speed varied between individuals.

Independent of forced swim trials, oxygen consumption varied independent of changes to water speed (Fig.5.1ii). These spontaneous increases in oxygen consumption linked to the locomotor activity of the fish as measured by the accelerometer in the form of VeDBA (Fig.5.1iia).

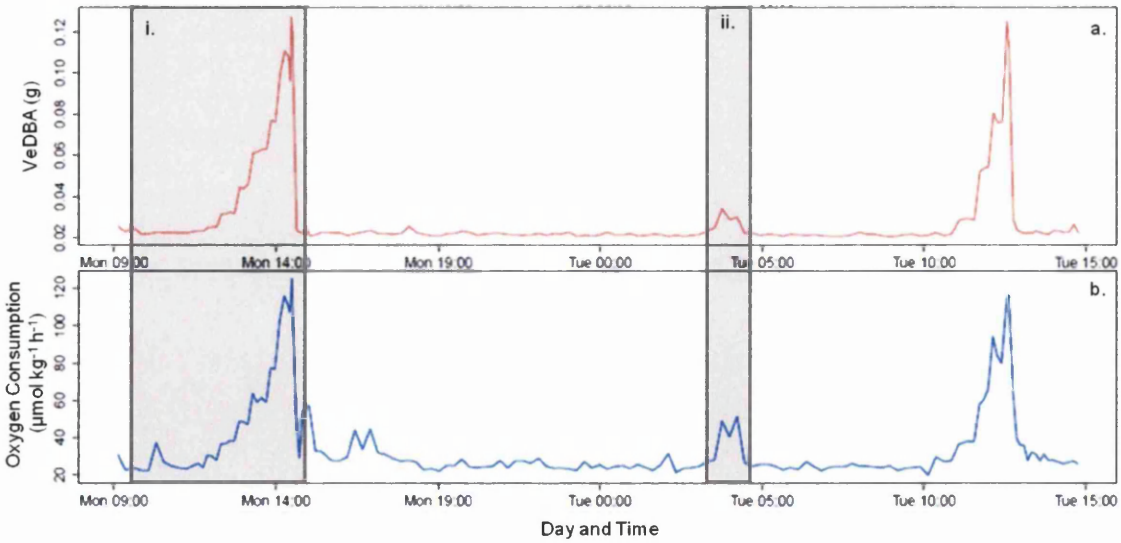

Figure 5.1. VeDBA, (a) and oxygen consumption (b) in relation to time for a European sea bass, highlighting a phase of steady swimming (i) and spontaneous activity (ii) within a swim tunnel respirometer.

4. Data Analysis

Data was processed and analysed using IgorPro (WaveMetrics, Inc, Oregon) and R (R Foundation for Statistical Computing, Vienna, Austria).

4. i. Preliminary processing of acceleration

All three axes of acceleration were calibrated by slow rotation through 360 ^{o} at the end of the experiments and a time lag of between 4 and 8 seconds was corrected for in all axes. The time lag occurred due to a cumulative discrepancy between the true time and the time set by the accelerometer. To estimate overall activity level of the fish, dynamic acceleration was derived from the raw acceleration trace (Gleiss et al., 2011; Gómez Laich et al., 2011; Halsey et al., 2009). Previous studies have used overall dynamic body acceleration (ODBA) and vectorial dynamic body acceleration (VeDBA) as the dynamic acceleration metric. VeDBA was selected as the metric to estimate activity-specific energy use for sea bass in this study as VeDBA is less influenced by the alignment of the tag relative to the body axes of the animal (Qasem et al., 2012; Wright et al., 2014). To calculate VeDBA from the acceleration trace, the static component was first extracted using a two

second smoothing algorithm (Shepard et al., 2008). VeDBA was then calculated using the following equation:

$$VeDBA = \sqrt{{A_x}^2 + {A_y}^2 + {A_z}^2} \quad (1)$$

where A_x, A_y and A_z represent absolute dynamic acceleration measured on the X, Y and Z axes of motion, respectively.

4. ii. Estimating EPOC using acceleration and MO2

To calculate EPOC for each activity and M_{O_2} value, firstly the aerobic limits of all activity levels were estimated for each temperature. To estimate these aerobic limits, M_{O_2} was regressed against VeDBA during steady swimming (excluding all other M_{O_2} values):

$$M_{O_2} = a\,\mathrm{VeDBA} + b \quad (2)$$

where, M_{O_2} is oxygen consumption (μmol O_2 kg^{-1} min^{-1}), VeDBA is acceleration (g), a is the slope of the line and b is the y-intercept (indicating the Standard Metabolic Rate or SMR). The confidence limits of the steady swimming regression were used to identify the minimum M_{O_2} level at a given activity (VeDBA) level, providing a prediction of the M_{O_2} value during aerobic locomotion. This was used as the "cut-off" point, above which a repayment of EPOC was assumed.

For periods where M_{O_2} was higher than predicted (as estimated from eqn 2), EPOC was calculated from the point of excessive oxygen consumption to the point that M_{O_2} returned to aerobic limits (below confidence limits of eqn 2), also defined as the area between the predicted M_{O_2} and the observed M_{O_2} ("auc" function in the MESS package, R). To obtain the predicted M_{O_2} of the fish for the estimation of EPOC, previous studies use routine or resting metabolic rate (RMR) (Brett, 1964; Lee et al., 2003; Zeng et al., 2010). RMR does not take into account spontaneous activity of the fish during the recovery phase. Thus, incorrect estimates of RMR may result in an under or over-estimate of EPOC and recovery time as noted by Lee et al., (2003). The present study incorporates locomotion-induced M_{O_2} use during recovery,

in an attempt to increase the accuracy of EPOC estimates and estimates of the amount of time required for recovery.

In addition to calculating the amount of EPOC and the time required for M_{O_2} to return to aerobic limits, the activity level (VeDBA) of the preceding exercise phase was also identified to assess whether the intensity or the duration of activity affected the amount of EPOC. The activity level was defined as the maximum acceleration of the fish during the oxygen recording period. The maximum acceleration was used to estimate the amount of time that the fish spent burst swimming.

Statistical analysis and modelling

A linear mixed effects model was used to establish the interaction between EPOC, exercise duration and intensity and between EPOC and recovery time. Temperature was accounted for as a covariate and fish ID was a random effect. The model of best fit was selected based on Akaikes Information Criterion (AIC) (Sakamoto et al., 1986).

Results

For all fish there was an initial decline in M_{O_2} as a result of EPOC during capture and transport to the respirometer. Following this M_{O_2} stabilised to a relatively low level (Fig.5.2). Therefore, M_{O_2} and *VeDBA* recorded in the first 12 hours were removed from data processing.

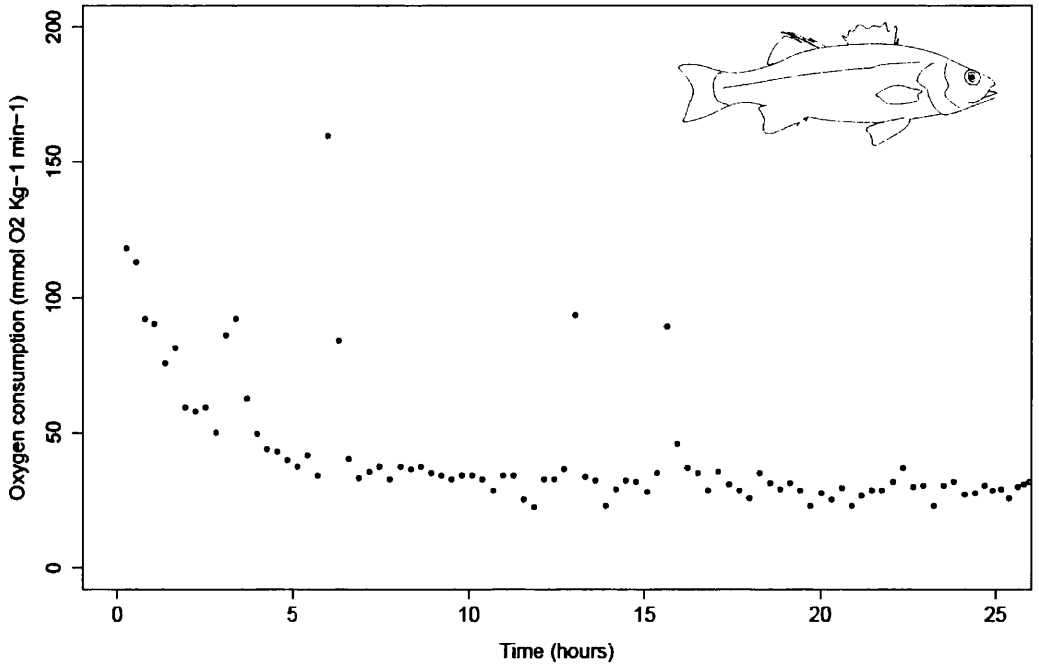

Figure 5.2. Raw M_{O_2} collected over 26 hours for a bass at 13.64 ± 0.03 ° C, swimming at 0.41 BL s^{-1}. Initial elevated M_{O_2} is caused by handling stress during capture and transport to the respirometer.

Prior to EPOC

EPOC ranged from 0 to 911.40 μmol O_2 (equivalent to 29.16 mg O_2), and was not significantly affected by temperature, but significantly increased with exercise intensity ($VeDBA_{Max}$) (P<0.0001) and exercise duration (P<0.0001). Thus, the model which fitted the data best incorporated exercise duration and maximal VeDBA:

$$EPOC = 0.28(Ex_{Dur}) \cdot 343.15(VeDBA_{Max}) - 34.19 \quad (3)$$

where, Ex_{Dur} corresponds to the exercise duration in minutes, and $VeDBA_{Max}$ is maximal VeDBA (g).

Post EPOC

Fish required a maximum of 70 minutes for oxygen consumption levels to return to aerobic limits. When EPOC was regressed against recovery duration, recovery time was the only significant variable (P<0.0001), with no significant effect of temperature (P=0.6766).Thus, all temperatures were combined for the final model (Fig.5.3), whereby:

$$EPOC = 7.752(EPOC_{Rec}) - 0.448 \quad (4)$$

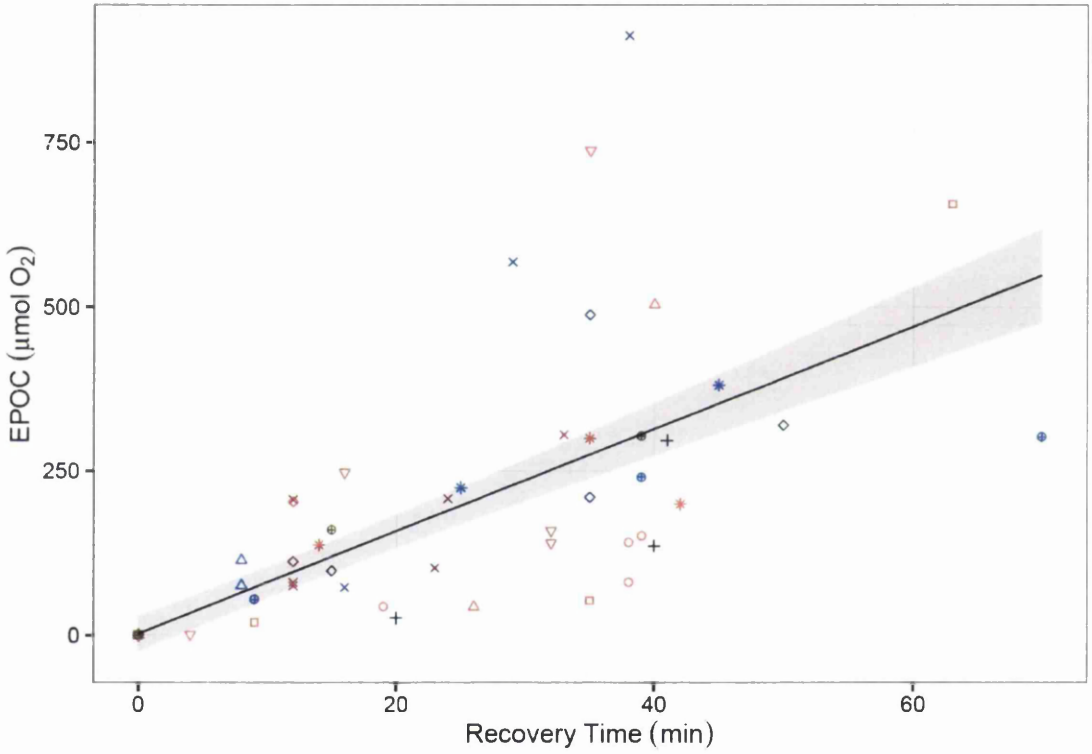

Figure 5.3. Excess post-exercise oxygen consumption (EPOC) in relation to the amount of time required for oxygen consumption to return to within aerobic limits for European sea bass, *Dicentrarchus labrax*, (individuals shown as symbols) at temperatures between 5 °C and 18 °C. The line corresponds to the linear mixed effects model between the recovery time and EPOC including the confidence limits of the model (Eqn 4).

Discussion

EPOC and activity intensity and duration

For sea bass in the present study, EPOC significantly increased with exercise intensity and exercise duration. When absolute values of EPOC are compared, our estimates (< 29.16 O_2 kg^{-1}) are lower than previously reported values for other teleosts including juvenile rainbow trout (>240 mg O_2 kg^{-1}) (Scarabello et al., 1992) and adult sockeye salmon (>61 mg O_2 kg^{-1}) (Lee et al., 2003). The relatively low EPOC estimates during this study may be due to methodological differences, including the technique used to induce EPOC. Previous studies either use short-high intensity exercise (Hyndman et al., 2003) or longer duration ramped swimming tests (U_{crit}) (Peake and Farrell, 2004; Scarabello et al., 1992) to induce exhaustion. In contrast, fish in the present study reached EPOC at a number of activity levels, with the intensity and duration of activity assessed for each temperature group.

When all results were compared, EPOC scaled positively with exercise intensity and duration, mirrored in results for humans (Bahr et al., 1992; Laforgia et al., 2006), horse (Langsetmo and Poole, 1999) and reptiles (Hancock and Gleeson, 2002). The increase in EPOC with exercise intensity can be independent of exercise duration (Langsetmo and Poole, 1999), though studies involving ectotherms including the desert iguana, *Dipsosaurus dorsalis* (Hancock and Gleeson, 2002) show an increase in EPOC with both intensity and duration, highlighting the importance of both short-high intensity exercise and long-low intensity exercise on energetic costs.

Increases in M_{O_2} were assumed to reflect increases in activity-specific energy use rather than stress. However, fish were held in enclosed tanks, which may result in increased oxygen consumption independent of activity (Bonga, 1997). The correlations between EPOC and exercise intensity and duration suggest that increases in energy use were induced by locomotion rather than stress for sea bass in this study.

In addition to activity level, temperature can affect the energy budgets of ectotherms like teleost fish (Clark et al., 2010; Schurmann and Steffensen, 1997; Wright et al., 2014). For example, EPOC significantly increases with temperature for

salmon (Brett, 1964; Lee et al., 2003) and catfish (Zeng et al., 2010), though, Zeng (2010) also found a decrease above 20°C. The initial increase in EPOC may relate to temperature-specific muscle performance (Guderley, 2004; Rome et al., 1985), with a greater anaerobic effort required for white muscles as temperatures increase (Kieffer et al., 1994), resulting in a concurrent increase in EPOC. There was no significant correlation between EPOC and temperature for bass in this study, potentially reflecting the dominant influence of the exercise intensity and duration on EPOC rather than temperature.

Recovery from EPOC

The amount of time that fish require to recover from EPOC has implications for their survival and fitness. During recovery, the amount of energy available for other behaviours, such as locomotion, may be reduced. Therefore, it pays for recovery to be as rapid as physiologically possible. For sea bass in the present study, the maximum recovery time was 70 minutes, which is similar to recovery times reported for wild adult sockeye salmon (48-78 min) (Lee et al., 2003) and wild adult cod (90 min) (Reidy et al., 1995), though is relatively short compared to juvenile sockeye salmon (192 min) (Brett, 1964) and juvenile rainbow trout (> 120 min) (Scarabello et al., 1992). The similarities in recovery time for bass in the present study to other adult teleost fish is surprising when the exercise intensity is compared; as the experiments with sockeye and cod involved the induction of maximal activity, either through increases in water speed or fish being chased to exhaustion, which theoretically should increase the required recovery time compared to fish in this study where fish were predominantly swam at sub-maximal activity levels. The relatively long recovery time for bass in the present experiment may relate to differences in physiological capacity, due to the prolonged period that the bass were kept in captivity prior to the start of the respirometry experiments (>10 years). Previous studies have shown that fish swimming performance can be affected by whether the fish are from the wild or aquaculture. For example, (Gamperl et al., 2002) found that the aerobic scope of hatchery reared rainbow trout was a third that of wild caught trout. The relatively long recovery time for sea bass in this study may

therefore reflect the long period of intensive rearing and thus a lack of exercise compared to wild stocks.

When overall trends between recovery time and EPOC are compared, there is a significant increase in the time required to recover when EPOC is greater and there is no effect of temperature. The positive relationship between EPOC and recovery is also apparent in adult sockeye salmon (Lee et al., 2003), reflecting the increased energetic costs and repayment required for higher EPOC. Previous studies have also found a temperature effect on recovery time in catfish (Zeng et al., 2010), with the longest recovery time required at 20°C, and reduced recovery times required at temperatures below and above this. The difference in recovery time with temperature for the catfish experiment may reflect the peak in EPOC at 20°C rather than a direct effect of temperature. In fact, there may be expected to be a faster recovery rate for muscle ATP and glycogen with temperature (Kieffer et al., 1994), with the increased metabolic capacity to clear EPOC with temperature (Jain and Farrell, 2003).

Methods

Previous studies have estimated EPOC after U_{crit} experiments or after fish were chased to exhaustion, enabling researchers to obtain a maximum estimate of EPOC. The choice of method to induce maximal EPOC significantly effects the post-exhaustion oxygen use (Reidy et al., 1995) and may also result in an overestimate of EPOC as energy use may involve both locomotor effort and stress induced costs from novel surroundings or air exposure. For example, (Scarabello et al., 1992) noted that the rate of recovery of rainbow trout was faster in the second run of the experiments, which was suggested to relate to a reduction in stress and the increased familiarity with the exercise protocol, as similarly noted by (Woodward and Smith, 1985).

We have increased the accuracy of calculating EPOC by using activity-specific M_{O_2} as the base line rather than a fixed routine or resting metabolic rate (RMR) estimate (Brett, 1964; Lee et al., 2003; Zeng et al., 2010). By incorporating minor changes in activity during recovery, estimates of recovery duration and EPOC may improve the accuracy of EPOC estimates.

Conclusion

Accelerometer data loggers can be used to estimate exercise intensity ($VeDBA_{Mean}$) and duration, which can then be used to predict the amount of EPOC at a given activity level and temperature, and thus the recovery time. The correlations between EPOC and EPOC duration, intensity and recovery may enable us to estimate total energy use from both aerobic and anaerobic locomotion of fish in the field. Further studies are required to assess scaling relationship between pre-EPOC behaviour and post-EPOC behaviour and recovery for other species of fish.

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Chapter 6

Changes in VeDBA with temperature for geographically distinct Atlantic cod populations

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S.R. Wright was responsible for data collection, analysis and writing the manuscript. Additional data was collected by Ø. Aas-Hansen. J. Metcalfe proof read the manuscript.

Abstract

Cod can experience rapid changes in temperature of as much as 6 °C in their natural environment, but the energetic and behavioural implications of these changes are difficult to quantify. The effect of temperature change (between 4 °C and 16 °C) on locomotor activity was investigated in Atlantic cod (*Gadus morhua* L.) from two geographically distinct populations (Southern North Sea and Northeast Arctic). In addition to the response after acclimation, the locomotor activity during the abrupt change to each new temperature was also assessed. Tri-axial accelerometers were used to measure the activity level of the fish (modal Vectorial Dynamic Body Acceleration) with temperature. Activity level significantly changed with ambient temperature for 60% of fish, with the temperature at maximal activity significantly higher for fish from the Southern North Sea (SNS) compared to the Northeast Arctic (NEA). The behavioural response of fish to abrupt temperature change was assessed by comparing the activity between the days leading up to the temperature transition with the day after. When responses were combined from the SNS and NEA stocks there was a significant reduction in activity the day after the change for 64% of the fish, whether the temperature was increased or decreased. In addition to providing useful insights into the behavioural responses of cod to temperature change, the present study highlights the application of accelerometers as a tool for indirectly monitoring the activity of fish in response to environmental change with the potential for use in the field.

Introduction

Atlantic cod, *Gadus morhua*, have a pan-Atlantic distribution and can inhabit waters from -1 °C to over 20 °C (Righton et al., 2010; Sundby, 2000). This has been linked to the thermal structure of the waters they inhabit (Jean, 1964; Perry and Neilson, 1988) with recent studies showing that in addition to seasonal fluctuations in temperature, cod can experience temperature changes of up to 6 °C as they travel across thermal fronts (Righton et al., 2010). The metabolic costs associated with coping with these changes in ambient temperature play a key role in the dynamic energy budgets of fish (Claireaux et al., 2006; Schurmann and Steffensen, 1997), with the rate and scale of ambient temperature change (absolute and abrupt) having physiological (Claireaux et al., 1995; Pérez-Casanova et al., 2008), behavioural (Fraser et al., 1993) and fitness implications.

Initial experiments looking into the effects of abrupt temperature change on fish, assessed mortality rate after exposure to rapid changes in temperature (Cherry et al., 1977; Currie et al., 1998), with more recent research showing sub-lethal impacts (Donaldson et al., 2008), including increases in oxygen consumption and heart rate (Claireaux et al., 1995) and decreases in locomotor activity (He, 1991). The decrease in locomotor activity associated with temperature change may be attributed to a reduction in the metabolic scope of the fish at times when temperature associated energetic costs are elevated (Claireaux et al., 2000) as the metabolic or aerobic scope corresponds to the difference between standard and active metabolic rate during aerobic locomotion (aerobic scope: AS) or during aerobic and anaerobic locomotion combined (metabolic scope: MS).

The effect of temperature change on cod may also be effected by their origin, with geographically distinct populations varying in their core physiological function, including their haemoglobin structure (Petersen and Steffensen, 2003), cold adaptation (Lannig et al., 2004) and costs of locomotion (Herbing and Boutilier, 1996). Previous studies show that for cod from relatively southern latitudes (Scotian shelf or Southern North Sea) standard and active metabolic rates increase with water temperature up to the optimum (Claireaux et al., 2000; Schurmann and Steffensen, 1997). Differences between AS_{max} and MS_{max} may relate to a reduction in the aerobic potential of muscle with temperature (Rome et al., 1985) resulting in a

concurrent increase in endurance performance (U_{crit}) (Lurman et al., 2009; Sylvestre et al., 2007). In contrast, the amount of swimming sustained by anaerobic locomotion (U_{burst}) decreases with temperature (Sylvestre et al., 2007).

Electronic "archival" or "data-storage" devices equipped with tri-axial accelerometers provide a method for estimating activity-specific metabolic rate (Gleiss et al., 2010; Wilson et al., 2013; Wright et al., 2014) and fine-scale behaviours (Broell et al., 2013) of fish in the laboratory and the field. By comparing relative changes in locomotor performance with environmental change (such as temperature), it is possible to assess the behavioural response of fish to ecologically important events, such as gradual and rapid changes in environmental temperature. The effect of temperature on the behaviour of fish is of particular importance for species exposed to significant and dynamic fluctuations in temperature like the Atlantic cod (Righton et al., 2010).

In this study we assessed the effect of temperature change on the activity level of cod after an abrupt temperature change and after fish had time to acclimate. The behavioural response was compared for two distinct cod stocks from the Southern North Sea (SNS) and the Northeast Arctic (NEA). Comparisons of spontaneous activity level were made both within and between stocks to identify whether there were differences in activity level with temperature at the individual and population level.

Materials and Methods

Animals and Data Collection

We conducted three experiments on two stocks of Atlantic cod. The first two involved fish from the Southern North Sea and the third involved fish from the Northeast Arctic.

Southern North Sea Cod (SNS)

SNS Atlantic cod were caught off the coast of Eastbourne (50.768 °N, 0.328 °E) in March 2011. After capture, fish were transported to the Cefas laboratory, Lowestoft and given a minimum of 6 months to recover from capture and transport before the start of the trial. The experimental tank for the behavioural trials measured 5 m × 0.95 m × 2.5 m (8.8 m^3). All fish were fed sand eel and sprat *ad libitum*. Fish were kept at ambient water temperatures.

Northeast Arctic Cod (NEA)

NEA Atlantic cod were caught off the coast of Northern Norway (~69 °N, 19 °E) in June 2013. After capture, fish were transported to Nofima laboratory, Trømso and given 2 weeks to recover from capture and transport. The experimental tank for the behavioural trials measured 2 m × 2 m (6.28 m^3). During trials, all stocks were fed a diet of sand eel, sprat and pellets *ad libitum*. NEA experiments were carried out between June and September 2013. Trials involved ten fish which were tagged on 4th and 5th June 2013.

Tag Specification and Surgical Procedure

All fish were tagged with G6a data storage tags (Cefas Technology; dimensions: 40 mm × 28 mm × 16.3 mm, 18.5g in air, 6.7g in seawater), which record acceleration in the three axes of motion corresponding to the surge (anterior-posterior), heave (dorso-ventral) and sway (side to side) motion of the fish. Tags were inserted into the body cavity of the fish using the method described in chapter 4. Briefly, tags were encased in medical grade surgical mesh (Polytetrafluoroethylene; Textile Development Associates, Inc., USA) or medical grade silicone (734 Flowable Sealant; Dow Corning Corp.) before they were inserted into the peritoneal cavity via a small incision in the ventral surface. Tags were secured in place in two locations using sutures attached to the left flank of the fish. The incision was closed using interrupted sutures and a coating of antibiotic powder (Vetremox antibiotic powder with an Orahesive mix [50:50]). Fish were transferred

to a recovery tank where they were held in a stream of aerated sea-water until they regained equilibrium.

Processing Acceleration

Accelerometers record both static and dynamic acceleration. Static acceleration is governed by the orientation of the fish with respect to gravity, whilst "dynamic acceleration" is induced by muscle contraction (Gleiss et al., 2010). Dynamic acceleration has been used as a proxy for activity-specific metabolic rate (Gleiss et al., 2010; Wilson et al., 2013; Wright et al., 2014), and indicates the activity level of the animal. For the present study, we are interested in activity change in response to water temperature change; we therefore extracted dynamic acceleration using the smoothing method detailed in Shepard et al., (2008). A three second smoothing algorithm was calculated as appropriate for cod in this study.

The orientation of the tag was not aligned to gravity, so Vectorial Dynamic Body Acceleration (VeDBA) was used as the proxy for activity level rather than Overall Dynamic Body Acceleration (Qasem et al., 2012). VeDBA was calculated as $\sqrt{{A_x}^2 + {A_y}^2 + {A_z}^2}$, where, A_x, A_y and A_z correspond to the absolute dynamic acceleration of the fish on the surge, heave and sway axes of motion, respectively. To limit the amount of individual variability between animals, VeDBA values were normalised by dividing VeDBA by the maximum acceleration for each fish. To ensure that the maximum was indicative of the true maximum for each fish, the reproducibility of the maximum was assessed as the duration of recording increased from 1 hour to 180 hours.

Tags were set to record acceleration for one hour per day for the duration of the study (due to a limited memory capacity) fish were fed 30 minutes before and 30 minutes after the recording period to attempt to encompass the full spectrum of fish motion (from low to high activity), as feeding can include excitability in anticipation of food (Brett and Zala, 1975), as well as suppressed activity in response to increased costs from postprandial energy use (Jobling, 1981).

Trends in activity with temperature and fish state

To identify whether activity level was affected by water temperature; trends between temperature and modal acceleration were assessed using polynomial regressions for each fish and for each stock (SNS and NEA). Regressions were fitted of increasing complexity (from 1 to 10 degrees), with the optima selected based on the minimum cross-validated squared errors of the fit against model complexity using Akaikes Information Criterion (AIC) and Bayesian Information Criterion (BIC).

Two-tailed t-tests were used to identify whether physical differences between fish influenced the significance of interactions between water temperature and activity. Total length, weight, pectoral fin length, stomach weight, gonad weight, liver weight, heart dimensions and condition factor were compared with the significance of regressions between water temperature and acceleration. Fulton's K condition factor was calculated as; $K_i = (\left(\frac{w_i}{l_i^3}\right) \cdot 100)$, where, w_i is the somatic weight (g) and l_i is the total length (cm) of cod i. K is indicative of the physiological state of the fish (Lambert and Dutil, 1997).

Fin clips were used to identify the haemoglobin genotype for the SNS and NEA stock (Barlow & Berenbrink, University of Liverpool, *pers. comm.*). Briefly, (1) DNA was extracted (2) PCR amplified and purified (3) and the genotype was determined by identifying polymorphic sites.

The temperature at the maximal activity level, $T_{max,A}$ for each fish was compared between SNS and NEA stocks using a two-sample t-test, with variance between groups derived using Bartlett's test of homogeneity (R Core Team, 2013).

Transitions in temperature

For experiment 2 (SNS) and experiment 3 (NEA) water temperatures were abruptly changed (by 4 °C) on at least one occasion and were kept at the new temperatures for 3 weeks (16, 12 and 8 °C and 4, 8 and 12 °C for experiment 2 and experiment 3 respectively). The behavioural response to each temperature transition was assessed by comparing the activity level the day after the temperature change

with the activity level leading up to and following the change. The significance of differences between activity levels was assessed using one-tailed t-tests.

Results

The total length (TL) of cod from the SNS ranged from 59.1 cm to 84.3 cm and for NEA cod from 64.0 cm to 83.0 cm (Table 6.1). Cod from the NEA had HbI-2 (70 %) or heterozygous (30 %) haemoglobin genotypes. We were unable to obtain haemoglobin types for three individuals from the SNS, though the remainder were heterozygous (50 %), with one HbI-1 and one HbI-2 cod. When characteristics of the stocks were compared, there was no significant difference in total length (t=-1.55, P=0.14), total weight (t=0.82, P=0.42), liver weight (t=0.44, P=0.66) or heart weight (t=0.93, P=0.37), though, there was a significant difference in gonad weight (t=3.43, P=0.007) and Fulton's K condition factor (t=3.04, P=0.007) between stocks.

Trends in activity with temperature and fish state

Activity (modal acceleration) was regressed against water temperature using the optimal polynomial regression calculated for each fish (Fig.6.1). There was a significant correlation for 7 fish from the SNS and 5 fish from the NEA using a first or second order polynomial regression.

Table 6.1. Individual fish characteristics for Atlantic cod (*Gadus morhua*), showing fish total length (TL), weight (W), sex, gonad weight (g) and Fulton's condition factor (K).

Stock	ID	TL (cm)	W (g)	Sex	Gonad weight (g)	K (g cm^3)	Haemoglobin Genotype
SNS	1a	59.20	2486	F	468	1.20	-
	1b	72.80	3362	F	432	0.87	Hbl-1/2 (Het)
	1c	65.50	3077	M	32.9	1.09	-
	1d	70.60	2969	M	17.3	0.84	Hbl-1/2 (Het)
	1e	59.10	1998	M	9.5	0.97	Hbl-1/2 (Het)
	2a	77.00	5576	F	1057	1.22	Hbl-1/2 (Het)
	2b	84.30	9010	F	1485	1.50	-
	2c	67.40	4729	F	831	1.54	HbI-1/1
	2d	78.20	5887	F	1296	1.23	Hbl-2/2
	2e	78.10	6996	F	931	1.47	Hbl-1/2 (Het)
$\bar{x} \pm se$		71.22±2.67	4609±712		656±172	1.19±0.08	
NEA	3a	78.00	5350	F	135	1.13	Hbl-2/2
	3b	79.00	4185	M	95	0.85	Hbl-1/2 (Het)
	3c	64.00	1975	F	11	0.75	Hbl-2/2
	3d	82.00	5505	F	85	1.00	Hbl-1/2 (Het)
	3e	69.00	2335	F	23	0.71	Hbl-2/2
	3f	76.50	4665	F	114	1.04	Hbl-2/2
	3g	81.50	3310	M	15	0.61	Hbl-2/2
	3h	76.00	4565	F	77	1.04	Hbl-1/2 (Het)
	3i	74.00	4425	F	63	1.09	Hbl-2/2
	3j	83.00	3155	M	24	0.55	Hbl-2/2
$\bar{x} \pm se$		76.30±1.90	3947±381		64±14	0.88±0.07	

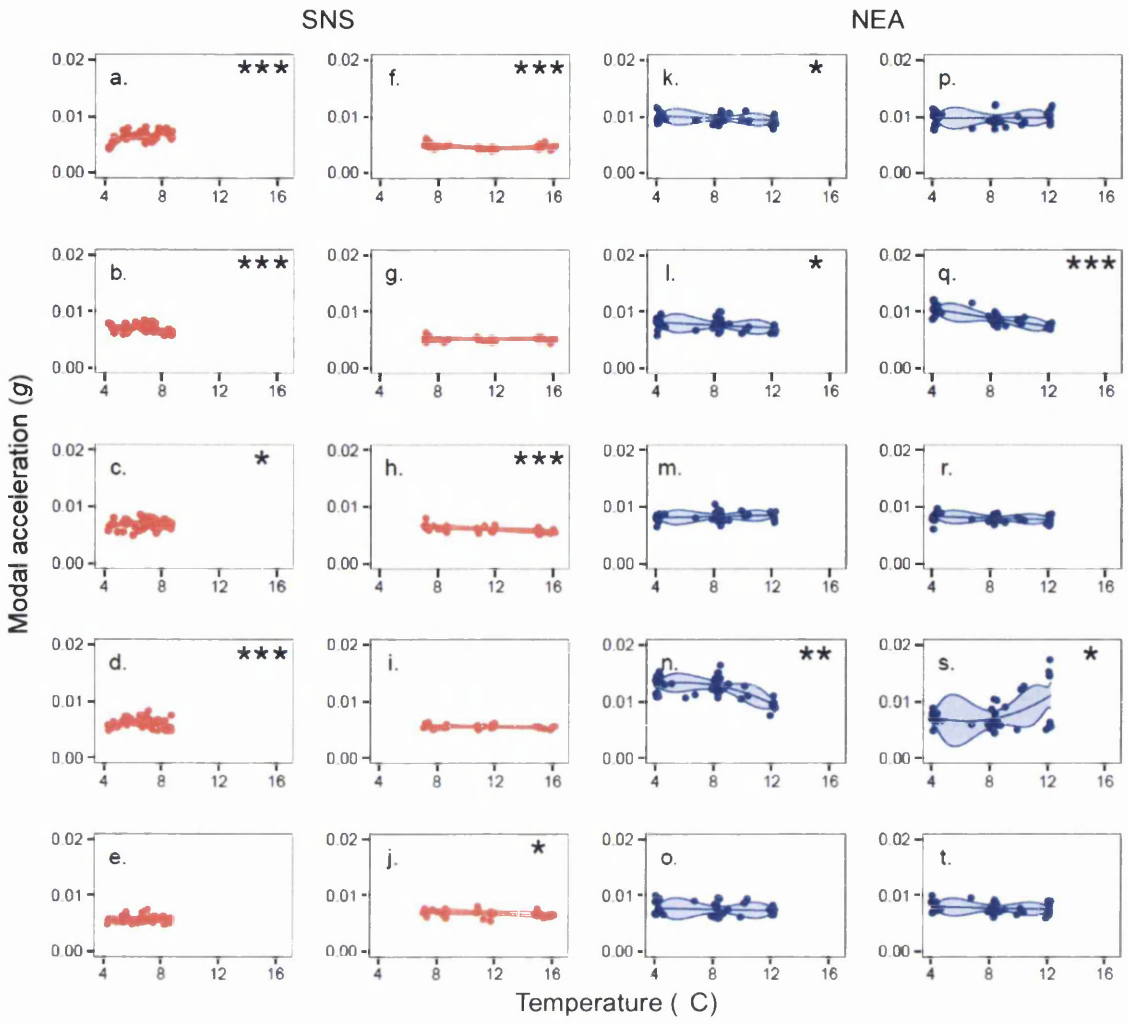

Figure 6.1. Activity (modal acceleration) in relation to water temperature for Atlantic cod from the Southern North Sea (a:j) and Northern North Sea (k:t). Regressions were fitted to each dataset based on the optimal polynomial fit, with the shaded area representing the 95 % confidence limits of the fit. Significance levels of fits are denoted by the number of stars in the upper right of each plot (* = 0.05, ** <0.01, * < 0.001). Plots without stars show no significant trend.**

The temperature at maximal activity was significantly higher for fish in the SNS compared to the NEA (t=5.1053, df=9, P<0.001). Using statistically significant regressions only, the temperature at the maximal activity level ($T_{max,A}$) for each fish was compared between the SNS and NEA using data from fish that had similar temperature experiences (Fig.6.3). There was no significant difference in the variance between the SNS and NEA stocks (Bartlett's K^2=0.77, P=0.38), so a t-test with equal variance was used to compare the stocks.

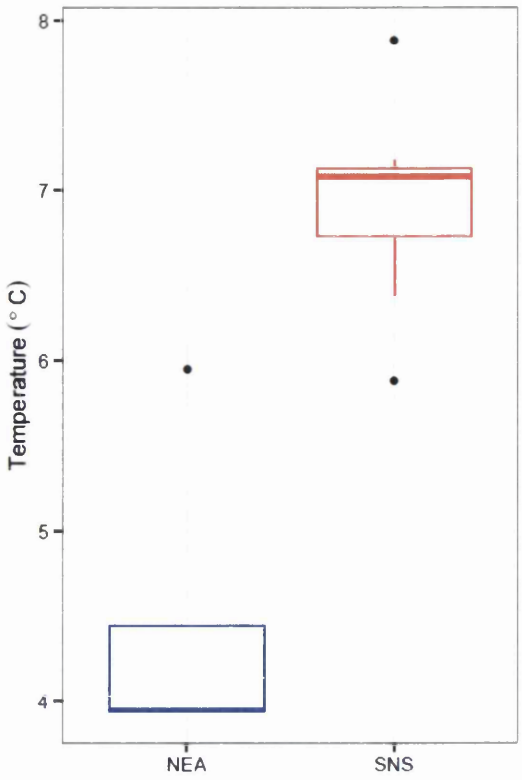

Figure 6.2. Temperature at maximal ($T_{Max,A}$) activity for Atlantic cod from the Northeast Arctic (NEA) and the Southern North Sea (SNS). Maximums were derived from fish with a significant correlation between temperature and activity (Fig.6.1). Using a two-tailed t-test, the temperature of maximal activity was significantly higher for fish from the SNS stock compared to the NEA ($t = 5.1053$, $df = 9$, $p < 0.001$).

The significance of interactions between water temperature and activity level was compared with individual characteristics using a two-tailed t-test. There was no significant difference ($P > 0.05$) between significant and non-significant results for any of the fish characteristics (TL, TW, Liver weight, Heart weight, *K*).

When all activity levels were combined for fish from the SNS, there was a significant correlation between modal acceleration and temperature ($F_{(4,985)} = 53.98$, $P < 0.0001$) using a fourth degree (quartic) polynomial regression (Fig.6.4a). All terms within the model (intercept, t, t^2, t^3 and t^4) were independently significant ($P < 0.0001$):

$$Acc_{mod} = 0.00649 - 0.00923(t) - 0.0039(t)^2 + 0.0071(t)^3 - 0.0036(t)^4$$

where, t is the temperature in °C. Notably, the temperature with the highest range of activity (difference between upper and lower quartiles) was at 4 °C for the SNS stock.

There was also a significant trend between the modal acceleration and ambient temperature for NEA cod ($F_{(1,421)}$= 4.541, P=0.034) using a first degree (linear) polynomial regression (Fig.6.4b). All terms (intercept and t) were independently significant in the model (P<0.05):

$$Acc_{mod} = 0.00885 - 0.00437(t)$$

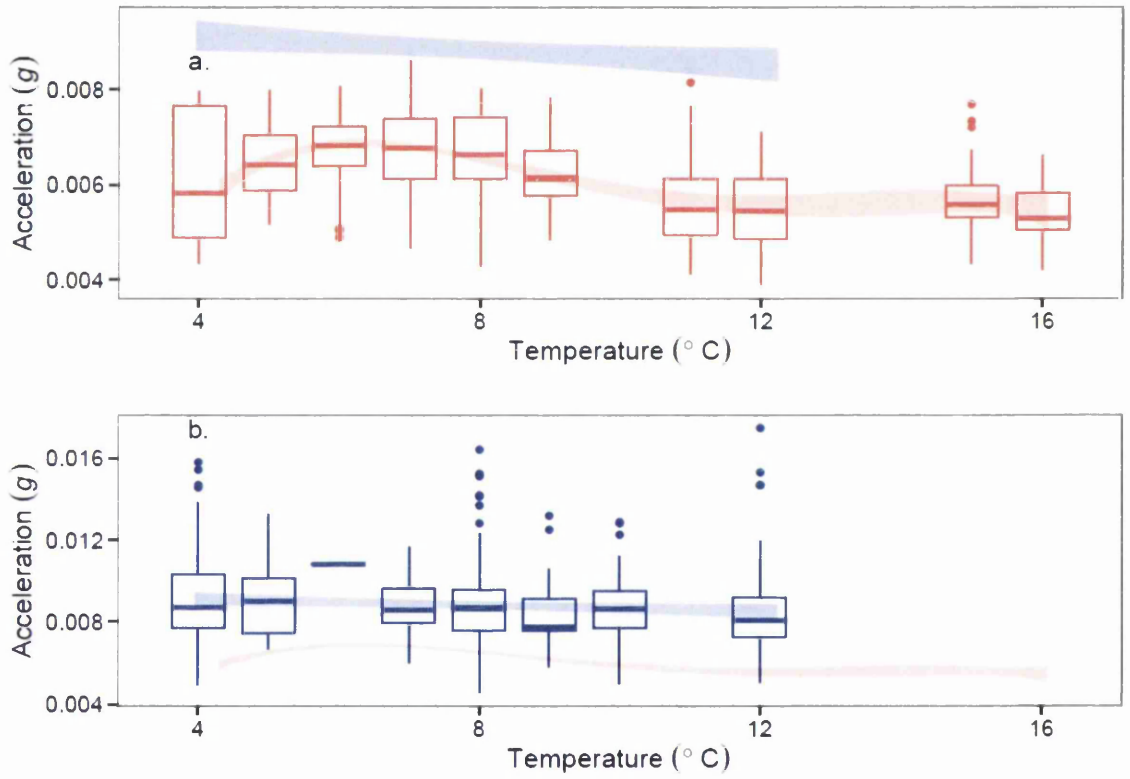

Figure 6.3. Effect of temperature on modal acceleration for Atlantic cod from the SNS (a.) and NEA (b.). The shaded areas correspond to the optimal polynomial fit for the two datasets with 95 % confidence intervals (fits for both stocks are shown in a. and b.). A fourth order polynomial (quartic) was most appropriate for the SNS stock and a first order polynomial (linear) for the NEA stock. Each boxplot shows the distribution of the data median (solid centre line), upper and lower quartiles (box extremities) and the outliers (individual points). Note parts a and b have different y axes.

Transitions in temperature

A one-tailed *t*-test was used to compare the activity level of fish in the days leading up to the transition with the activity the day after. When all transitions for fish from the SNS and NEA were combined, 64 % of fish had significantly lower activity levels the day after the transition, one had significant higher and 32 % showed no significant change (Table 6.2).

A one-tailed *t*-test was used to compare the activity level after the fish had acclimated to the tank (between 5 and 20 days after the transition). 36 % of fish had significantly lower activity level the day after the temperature change compared to after the fish had time to acclimate. There was no significant difference in activity level for 48% and activity was significantly higher for 16 % (Table 6.2).

Table 6.2. Comparison of activity level ($VeDBA_{Mode}$) in the time leading up to a temperature change with the day after the change for cod from the southern North Sea (SNS) and Northeast Arctic (NEA). Letters denote whether the activity was lower (L) the day after the change or higher (H). Letters in bold signify a significant difference.

Stock	Temperature change (°C)	ID	Before	After
SNS	16 to 12	2a	**L**	**L**
		2b	**L**	L
		2c	L	L
		2d	**L**	**L**
		2e	**L**	**L**
NEA	4 to 8	3a	**L**	L
		3b	**L**	**L**
		3c	**L**	L
		3d	L	**H**
		3e	**L**	L
		3f	**L**	L
		3g	L	L
		3h	**L**	L
		3i	**L**	**L**
		3j	**L**	**L**
	8 to 12	3a	L	L
		3b	**L**	**L**
		3c	L	=
		3d	H	**H**
		3e	**H**	**H**
		3f	**L**	**L**
		3g	**L**	**H**
		3h	L	H
		3i	**L**	**L**
		3j	L	L
Proportion of significant differences (%)		L	64	36
		H	04	16
		None	32	48

Discussion

Acclimated temperature response

Locomotor activity (modal VeDBA) varied with temperature at both the individual and stock level, with a significant change in activity for 60% of fish. The temperature at maximal activity ($T_{max,A}$) was 6.9 °C for SNS cod and 4.5 °C for NEA cod. Activity levels were reduced at temperatures below and above $T_{max,A}$ for the SNS stock, with a reduction in activity above $T_{max,A}$ for the NEA stock. The temperature at maximal activity reflects the temperature at which cod were willing to expend the most energy which may correspond to the temperature that the fish has the largest scope for activity (metabolic scope). Cod in the present study were able to use both aerobic and anaerobic locomotion, so $T_{max,A}$ more likely corresponds to MS rather than AS.

Previous studies show that for cod from relatively southern latitudes (Scotian shelf or Southern North Sea) standard and active metabolic rates increase with water temperature up to the optimum (Claireaux et al., 2000; Schurmann and Steffensen, 1997), with aerobic scope maximal (AS_{max}) at 10 °C (Claireaux et al., 2000) and metabolic scope maximal (MS_{max}) at 5 °C (Schurmann and Steffensen, 1997). Differences between AS_{max} and MS_{max} may relate to a reduction in the aerobic potential of muscle with temperature (Rome et al., 1985) resulting in a concurrent increase in endurance performance (U_{crit}) (Lurman et al., 2009; Sylvestre et al., 2007). In contrast, the amount of swimming sustained by anaerobic locomotion (U_{burst}) decreases with temperature (Sylvestre et al., 2007). Thus, for cod in this study, as temperature decreased there may have been a shift from aerobic to anaerobic locomotion, resulting in increased activity at lower temperatures. Notably, at the lowest recorded temperature for the SNS stock (4 °C) the median VeDBA was relatively low with a large range in activity levels; potentially reflecting fish using a mixture of low activity and burst locomotion rather than solely aerobic steady locomotion.

Haemoglobin genotypes were compared between stocks as cod populations consist of two homozygous genotypes (HbI-1 and HbI-2) and one heterozygous genotype (HbI-1/2). Cod were predominantly heterozygotes in the SNS and HbI-2 in

the NEA. Haemoglobin genotypes affect the preferred temperature of cod (Petersen and Steffensen, 2003) due to differences in physiological processes between stocks (Jobling, 1981). The lower $T_{max,A}$ of the NEA stock compared to the SNS stock may reflect cold adaptation in the white muscle of the NEA fish, as Northern stocks have higher activities of cytochrome *c* oxidase and citrate synthase in white muscle compared with southern populations (Lannig et al., 2004). However, the present study was limited to temperatures between 4 °C and 16 °C. Thus, for the NEA stock, the temperature at maximal activity was at the lowest experimental temperature, potentially resulting in $T_{max,A}$ being an overestimate. Future studies should assess the behavioural response of cod to temperatures below 4 °C, as the temperature range that the two stocks experience in the field varies from 2.32-19.45 °C for cod from the SNS compared to -1.54-11.71 °C for cod from the Barents Sea (Righton et al., 2010). Thus, we may anticipate that the lower temperature limit for cod from northern latitudes will result in a lower $T_{max,A}$ than reported in the present study.

When overall activity levels are compared between stocks, fish from the NEA were more active than the SNS, which may indicate regional adaptation. Herbing & Boutilier (1996) found that the metabolic cost of activity was significantly higher for cod from the Scotian Shelf (44 °N) than cod from Newfoundland (47 °N), reflecting an increase in cost of locomotor activity for cod at lower latitudes. Thus, the lower activity level of the SNS stock in this study may reflect a higher energetic cost of locomotion compared to the NEA stock.

Fish of a similar size were used in this study; with no significant difference in lengths or weights of the SNS and NEA stocks. However, fish from the SNS had significantly higher gonad weight and condition compared to the NEA stock. Previous studies have noted that locomotor activity can be affected by condition in cod (Guderley, 2004; Lapointe et al., 2006; Martinez, 2003), herring and plaice (Blaxter, 1974). Martinez, (2003) suggests that reductions in locomotor activity may constitute a strategy to conserve energy when the condition of the fish is poor. Nevertheless, for cod in this study, differences in the condition and maturity did not affect their behavioural response to temperature.

Abrupt temperature response

Results from the present study imply that there were two dominant behavioural responses to abrupt temperature transitions; either, activity was not significantly affected, or activity was suppressed. Previous studies have defined a two phase response to abrupt temperature change for Atlantic salmon (*Salmo salar*) (Peterson and Anderson, 1969) and Atlantic cod (Claireaux et al., 1995), consisting of (1) a transient phase during the period of temperature change, and (2) a stabilisation phase after the temperature change. This behavioural response is suggested to reflect an escape response in stage 1 and physiological adaption in stage 2. The dominant response of fish to the temperature change in the present study was a reduction in locomotor activity mimicking the phase 2 responses for cod (Claireaux et al., 1995) and salmon (Peterson and Anderson, 1969). This reduction in activity level may reflect a means of conserving energy after incurring physiological costs from the abrupt temperature change which can include increases in heart rate (Claireaux et al., 1995) and cortisol production (Gamperl et al., 2009).

Findings from this study emphasise the impact of abrupt changes in water temperature on the behaviour of fish. To better understand these effects, future studies should focus on the behavioural response of fish during a temperature change to better understand the first phase of the behavioural response (stage 1).

Conclusion

Tracking the activity of fish enables us to quantify behavioural responses to biotic and abiotic changes in the environment. The effect of both abrupt and gradual temperature change on the activity of Atlantic cod was assessed for two geographically distinct populations. This study highlights the complex and dynamic responses individuals can have to thermally diverse habitats. The behavioural trends in activity with temperature are some of the first to be documented for fish in semi-free-ranging controlled conditions in the laboratory. The response of fish in the field may be very different as the complex and dynamic interactions of biotic and abiotic factors influence energetic budgets (Cote et al., 2002). Future studies should tag and

track behavioural responses of fish to temperature change of free-ranging individuals.

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Chapter 7

Preparing for attack: strike types and performance for a sit & wait predator

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S.R. Wright, T. Noda, F. Bröll, J. Johannsen, J. Steffensen and P. Domenici collected the data. S.R. Wright was responsible for the analysis and writing the manuscript. P. Domenici and S. Wright conceived the study and P. Domenici proof read and made amendments to the manuscript.

Abstract

Predator-prey interactions govern the fitness and survival of fish in the wild, yet are difficult to quantify due to the high speed at which they occur. High frequency tri-axial accelerometers provide a tool for effectively recording these events enabling us to explore the factors that influence outcomes. Differences in attack style and the performance of strikes was assessed for great sculpin (*Myoxocephalus polyacanthocephalus*) attacking sand eel (*Ammodytes spp.*) using both high speed cinematography and tri-axial accelerometry. Strikes had two main phases; (1) a preparatory phase whilst the sculpin was stationary and (2) a propulsive phase, involving the rapid acceleration of the sculpin towards the sand eel. At rest, prior to the strike, sculpin had a positive or negative bend in their tail. During the preparatory phase, the caudal fin slowly bent towards the convex (A) or concave (B) side of the body. Behavioural differences between strike types A and B were compared with strike success both within and between individuals to identify key factors affecting the success likelihood of strikes, which include the relative orientation and position of the prey to the predator. In addition to insights to strike behaviour of sculpin, the methods used in the present study can be used as a tool to better understand predator-prey interactions for other species.

Introduction

Predator-prey interactions are a critical component in our understanding of ecosystem dynamics. Predation leads to the removal of prey from ecosystems, with knock on effects not only to the prey population, but also the ecosystem as a whole. In addition to the effect of prey loss on ecosystems, the success and more importantly, failure of predation attempts can also have a critical effect on the ecosystem, with the risk of predator starvation possible in areas where prey are a limited resource. Studies have investigated both sides of these complex events (Turesson et al., 2004; Turesson et al., 2009), though there are still gaps in our knowledge.

Using fish as a model, predator-prey interactions can be monitored relatively easily within the confines of a tank. Within this model, one of the key factors affecting predator-prey interactions is the fast start, which enables fish to accelerate towards a prey or away from an attacking predator and involves the contraction of anaerobic glycolytic (white) muscle fibres over a fraction of a second (Domenici and Blake, 1997). The type of postural curvature achieved by the end of the first muscle contraction is often used to define the type of fast-start, with both C- and S-starts commonly defined and referred to in the literature (Domenici, (2011) for a review). C- and S-starts were initially thought to be used solely for escape or strikes, respectively, but insights from new studies highlight the flexibility in predator-prey behaviours. For example, feeding strikes of pike involve an S-shaped caudal bend (Schriefer and Hale, 2004) whilst feeding strikes of archer fish involve a C-shaped bend (Wöhl and Schuster, 2007).

In addition to the type of curvature at the end of the first muscle contraction, predation attempts are also influenced by a number of other factors specifically involving the behaviour and escape potential of prey and the attack strategies of predators (Rice and Hale, 2010)). For example, for sedentary or immobile prey, predator positioning is crucial to optimise the energetic cost of attacks (Vinyard, 1982). Predator position is also important when attacking evasive prey, but there are numerous other variables that can affect strike success, including prey density (Turesson et al., 2004) and orientation (Webb and Skadsen, 1980). Bearing this in mind, predators must adapt their behaviour in response to the manoeuvrability and

escape potential of their prey, with both non-locomotory (strike distance, prey position and orientation) (Harper and Blake, 1991), and locomotory (acceleration and speed) (Webb and Skadsen, 1980) variables affecting the outcome of strikes.

Non-locomotor variables relate to the behaviour and sensory strategies of predators. For example, predators can decide on the optimal timing of a strike to maximise the likelihood of success, utilising information about prey size, orientation (Webb and Skadsen, 1980), relative position (New et al., 2001) and distance (Domenici, 2002), all of which rely on the performance of sensory systems including vision (Grobecker, 1983) and the lateral line (Montgomery et al., 1997), alone or in combination (Liang et al., 1998; New et al., 2001). After predators are aligned and prepared for a strike, the locomotory phase begins which involves the predator accelerating towards the prey in a capture attempt. The locomotory phase is affected not only by acceleration, but also deceleration, speed and manoeuvrability of the predator (Gerstner, 1999; Nemeth, 1997). For example, the performance of a fast-start may be influenced by the degree of postural curvature prior to the strike, as noted for escaping prey (Turesson et al., 2009).

In addition to the effect of both non-locomotor and locomotor variables on the outcome of strikes, attack strategies must also account for the potentially low frequency of prey encounters and the costs associated with fast-starts of unsuccessful attempts.

The present study is one of the first to explore the behavioural strategy and performance of strikes for the sit-and-wait predator, the great sculpin, *Myoxocephalus polyacanthocephalus*, using both high speed video and tri-axial accelerometry. The combined use of these two technologies will provide detailed insight into these crucial strike events. Differences in behaviours are compared with strike success both within and between individuals to identify key factors affecting strike performance. We may anticipate that sculpin use similar attack types to other sit-and-wait predators like the stone fish and pike, which rely on both vision and their lateral line.

Materials and Methods

Experiments were carried out under the authority of the University of Washington in accordance with Institutional Animal Care and Use Committee standards (permit no. 4238-04).

Fish and Experimental Design

Six great sculpin (*Myoxocephalus polyacanthocephalus*) were collected using a beach seine at two locations on the southeast coast of San Juan Island, Washington, USA (48 °N, 123 °W). Fish were transferred to the Friday Harbor Laboratories where they were placed in a holding tank at 11 °C. Fish ranged in size from 29.0 to 35.0 cm fork length weighing between 560 and 940 g (Table 7.1). All equipment and tagging procedures are described in Broell et al., (2013). Briefly, acceleration was recorded using an ORI-380D3GT micro-accelerometer data storage tag (Little Leonardo, Tokyo, Japan), which measured 12mm × 45 mm and weighed 10 g in air. Tags were set to record tri-axial acceleration (± 4 *g*) at 100 Hz using 12 bit resolution. In conjunction with the accelerometer, a video recorder was set to record at 30 Hz using a standard USB webcam (Microsoft LifeCam, VX-1000 and H264 Webcam 3.83 software, Redmond, WA, USA) and at 500 Hz using a high-speed camera(GIGE Vision, Fastec Imaging HiSpec 2G Mono) positioned at 2.6 m above the tank bottom.

Table 7.1. Great sculpin total length (TL), weight (W), condition factor (*K*), ratio between attack type, ratio between outcomes and proportion of strikes resulting in success.

Fish	TL (cm)	W (g)	*K*	Type (A:B)	Outcome (S:F)	Proportion successful (%)
1	35.0	560	1.31	0:6	1:5	20.0
2	40.0	760	1.19	7:4	3:8	37.5
3	36.7	-	-	3:8	5:6	83.3
4	39.0	701	1.18	6:3	1:8	12.5
5	38.5	560	0.98	3:6	2:7	28.6
6	35.0	590	1.38	0:8	2:6	33.3

Prior to the start of a trial, sculpin were tagged with an accelerometer during the transfer from the holding tank (170 cm diameter) to the adjacent trial tank

(identical to holding tank), where water was maintained at 50 cm depth. Tags were attached to a piece of Velcro connected through the dorsal muscle of the fish (Fig.7.1a), which was applied prior to the start of the experiment. The transfer and tagging time took between 2 and 3min from tank to tank. Fish were given 30 minutes to recover from transfer and tagging before the start of the prey capture trial, after which, between one and ten sand eel (*Ammodytes* spp.) were introduced to the tank. The recovery time is relatively short compared to other tagging studies (Brownscombe et al., 2014), but was deemed sufficient, as sculpin were observed feeding within a few minutes of transfer to the experimental tank. By the end of the trials, between 12 and 22 feeding attempts (successful or not) were recorded for each fish using the accelerometer and video recorder. Timestamps on the accelerometer and video recording were used to match up fast-start events. Video footage was analysed frame-by-frame using WINanalyze (Mikromak GmbH), to automatically extract trajectories of the snout, midpoint and tip of the caudal fin of both the predator and the prey (Fig.7.2).

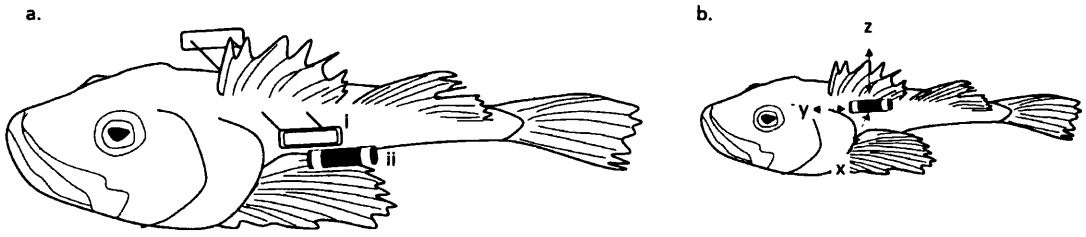


Figure. 7.1. Schematic diagram showing the attachment method for accelerometers to the dorsal muscle of the great sculpin, *Myoxocephalus polyacanthocephalus* (a). A Velcro attachment (i) is secured in place with pins through the dorsal muscle, the tri-axial accelerometer (ii) is then connected to the Velcro at the start of feeding trials. The accelerometers record acceleration on three planes of motion (b) corresponding the sway (x), surge (y) and heave (z) acceleration of the fish.

Geometry and Kinematics of the attacks

Five variables were used to characterise sculpin attack behaviour at the point of first motion (*Tt*): strike distance, duration, relative position, orientation and postural curvature. Strike distance was calculated from the tip of the snout of the sculpin to the centre of mass of the sand eel (Fig.7.3Ac), as the prey centre of mass is the point that moves least during escapes (Webb and Skadsen, 1980) and

corresponds to the point about which propulsive forces act (Webb, 1978b). The centre of mass was calculated as the point along the stretched straight length of a sculpin at which weight was equally balanced, and was calculated from a frozen sculpin specimen. Strike duration was the difference in time from the point of first motion (*Tt*) until the sculpin reached a point along the trajectory of the prey (the anticipated location of the prey). The relative position was the angle between the prey body axis and the strike path of the sculpin (Fig.7.3Ad), and ranged from 0 ° (prey directly in front of the predator) to 180 ° (prey directly behind the predator). Relative orientation was defined as the difference in the postural direction of the predator compared to the prey (Fig.7.3Ae), and ranged from 0° (body axes aligned in exact opposite directions) to 180° (body axes aligned snout to snout). Finally, the postural curvature of the sculpin was calculated as the absolute angle of the caudal fin at first motion (Fig.7.3Af), reflecting the degree of curvature of the body relative to the tail at the point of first motion.

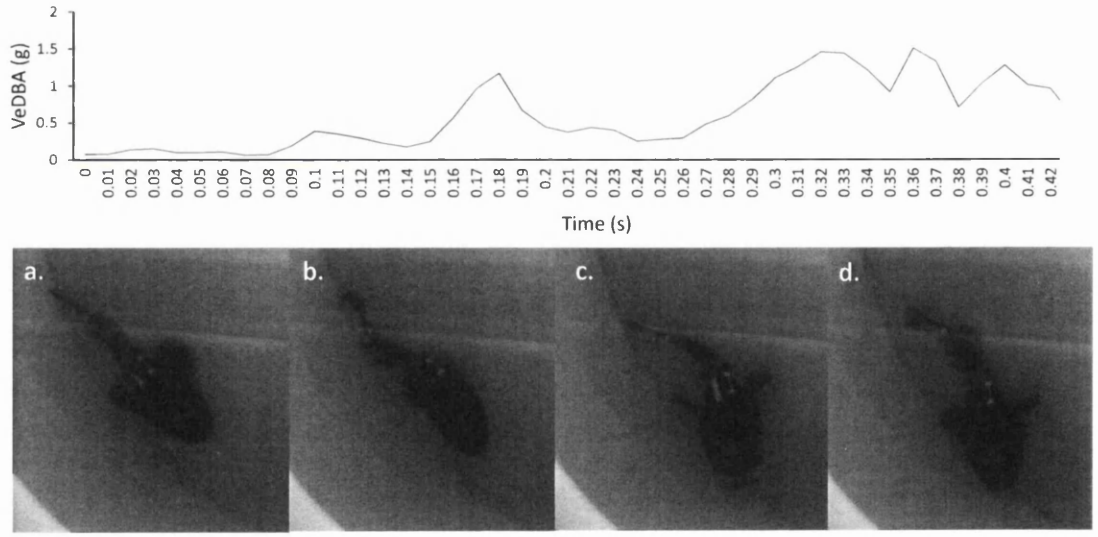


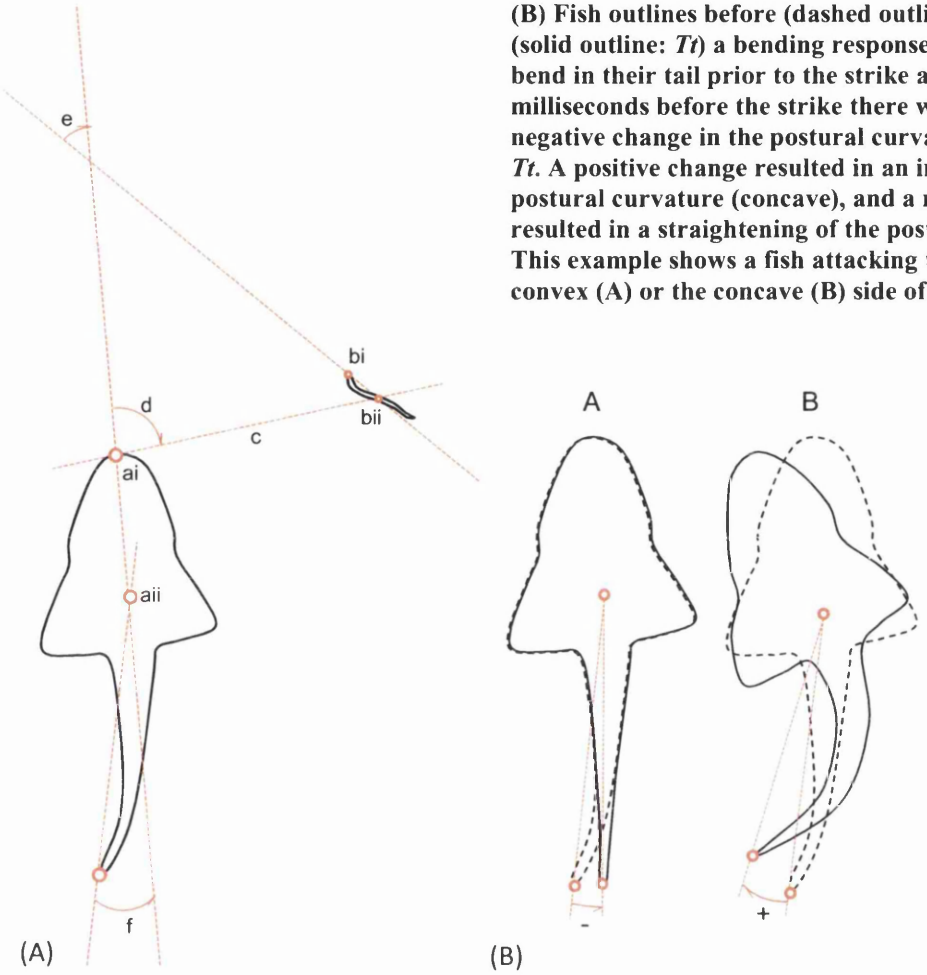
Figure 7.2. High-speed video footage showing a sculpin strike (a) before acceleration, (b) at the start of acceleration, (c) mid-strike and (d) after the strike. The corresponding vectorial dynamic body acceleration (VeDBA) is shown above the video stills.

Performance using accelerometry

In order to obtain an accurate measure of fish performance, both cinematography (Borla et al., 2002; Grobecker, 1983) and accelerometry (Broell et al., 2013; Harper and Blake, 1991) have previously been used. For sculpin in this study, strike performance was measured as the acceleration of the predator from *Tt* until the sculpin reached the anticipated location of the prey. Accelerometers record three-dimensional acceleration of strikes, providing a robust metric of acceleration for fish in a multi-dimensional environment. Furthermore, tri-axial acceleration has previously been linked to activity-specific energy use of a number of fish species including hammerhead sharks, *Sphyrna lewini* (Gleiss et al., 2010), sockeye salmon, *Oncorhynchus nerka* (Clark et al., 2010; Wilson et al., 2013) and sea bass, *Dicentrarchus labrax* (Wright et al., 2014). The vectorial dynamic body acceleration (VeDBA) was derived from the combined raw acceleration on all three axes of motion, VeDBA = $\sqrt{x^2 + y^2 + z^2}$, where, x is the sway axis, y is the heave axis and z is the surge axis of acceleration (Fig.7.1b). Strike performance was calculated as the mean ($VeDBA_{Mean}$) acceleration over the duration of the attack.

Figure 7.3. (A) The predator (a) and prey (b) snout (i) and centre of mass (ii). The strike distance between the predator snout and the centre of mass of the prey (c), the relative angular position of the prey to the predator (d), the relative orientation of the prey to the predator (e) and the postural curvature (f). The strike distance, relative position, relative orientation and curvature were measured at the point of first motion of the predator (*Tt*).

(B) Fish outlines before (dashed outline: Tt_{-1}) and after (solid outline: *Tt*) a bending response. Sculpin had a bend in their tail prior to the strike and in the milliseconds before the strike there was a positive or negative change in the postural curvature from Tt_{-1} to *Tt*. A positive change resulted in an increase to the postural curvature (concave), and a negative change resulted in a straightening of the posture (convex). This example shows a fish attacking towards the convex (A) or the concave (B) side of the body.



Statistics

Analysis of variance was used to assess whether there were significant differences between kinematic variables and strike performance for different strike types. Fish ID was applied as a covariate within the model to account for differences between individuals (covariance). Significance was set at 0.05.

Results

Sculpin remained stationary in the tank whilst prey were introduced in groups of between one and ten. After introduction, prey spent the majority of their time swimming near the walls of the tank with occasional ventures to the centre. When

prey came within close proximity of the sculpin, the eye movement of the sculpin suggested the predators were tracking the prey.

There were two stages to each strike; (1) a preparatory and (2) a propulsive stage. At rest sculpin had a positive or negative bend in their tail. The sculpin then elicit a bending response whilst stationary, which involved the slow, lateral movement of the caudal fin towards the convex (-: Type A) or concave (+: Type B) side of the body (Fig.7.2B). This phase occurred prior to the point of first motion and was calculated from the angle of change of the caudal fin between Tr^{-1} and Tt (Fig.7.2B). The second phase was the propulsive phase which involved the rapid motion of the caudal fin and the acceleration of the sculpin towards the prey. When strike type was compared between individuals, the two fish with the highest condition factor did not use type A strikes (Table 7.1).

Strike outcome

For sculpin in this study, we found no significant correlation between predator size and strike distance, suggesting that other factors had a greater effect on the outcome of the strikes. When the likelihood of success was compared between individuals, the proportion of strikes which resulted in success varied from 12.5 % to 83.3 % (Table 7.1). When the outcome of the strike was compared between attack types, type B attacks were more likely to result in success (41 %) compared to type A (22 %) (Table 7.2) and when all attacks were combined, the only significant factor that affected the outcome was the relative position of the prey (F=4.811, P=0.034) (Fig.7.7), with strikes more likely to be successful when the prey were positioned at <90 °.

Preparatory phase

During the preparatory phase, prior to the strike, the postural curvature of the sculpin was on average 12.25±1.27 ° with a significantly higher curvature for type B attacks compared to type A (F=6.767, P=0.013) (Fig.7.4a).

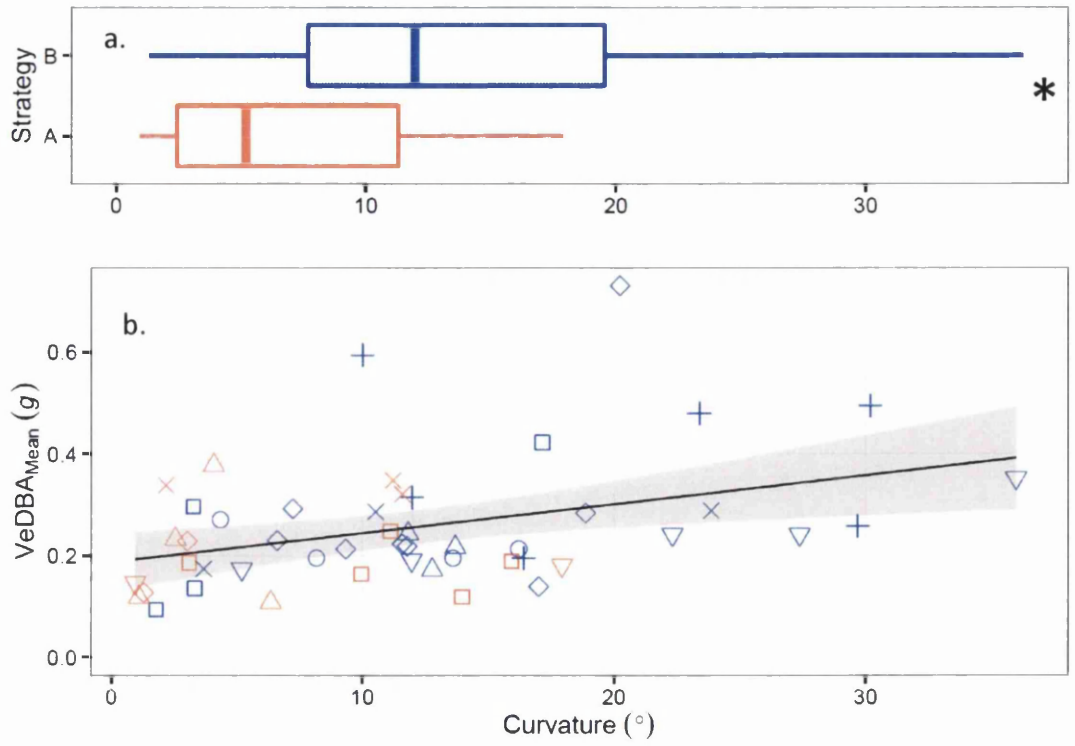


Figure 7.4. Box-whisker plot showing the significant difference in the absolute postural curvature between type A and type B attacks (a), and the absolute postural curvature in relation to mean vectorial dynamic body acceleration (VeDBA$_{Mean}$) for sculpin attacking using type A (orange) and type B (blue) attack types (b).

Strikes were initiated when prey were at a distance of between 6.1cm and 126.9cm and ranged in duration from 0.6 s to 3.8 s (Table 7.2). There was a significant correlation between strike duration and strike distance (F=23.174, P<0.001), with shorter attacks when prey were closer to the predator.

The relative position of the prey to the predator at first motion (Fig.7.2d) ranged from 20° to 180°, with a peak at +/-96 °, which corresponds to when the sand eel was within the visual field of the sculpin (Fig.7.5a). The relative orientation of the prey to the predator was between 5.48 ° (body axes of predator and prey aligned in similar directions) and 174.30 ° (body axes aligned in opposite directions), and was highest at +/- 97 ° (Fig.7.5b, Table 7.2), corresponding to when the sand eel were perpendicular in orientation to the sculpin.

Table 7.2. Kinematics and performance of type A and B attacks. Values are shown as mean ± standard error. Significant differences between attack types (A & B) are denoted by the preceding asterisk (*).

Type	Bending angle	Kinematics					VeDBA		Outcome	Proportion successful
		Distance (cm)	Duration (s)	Curvature (°)	Orientation (°)	Position (°)	Mean (*g*)	Max (*g*)	(S:F)	(%)
A	-	20.13±3.23	1.47±0.17	7.26±1.44*	95.35±10.89	93.47±12.93	0.20±0.02*	4.56±0.43	4:18	22.22
B	+	28.11±4.28	1.50±0.12	14.54±1.60*	100.14±8.89	96.61±7.65	0.27±0.02*	5.68±0.40	11:27	40.74
Combined	+ & -	25.50±3.10	1.49±0.10	12.25±1.27	98.61±6.92	95.61±6.57	0.25±0.02	5.26±0.30	15:45	33.33

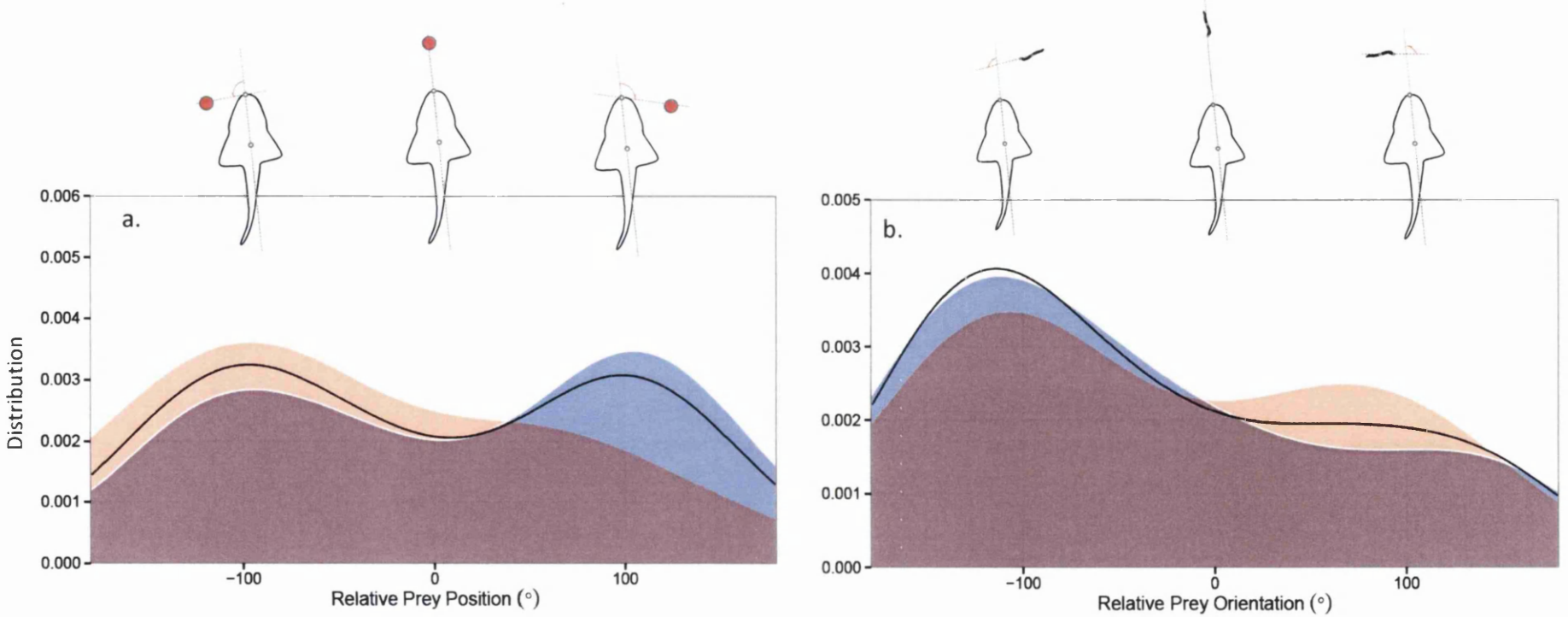


Figure 7.5. Relative position (a) and orientation (b) of the prey to predator at the point of first motion for type A (orange) and type B (blue) attack types, and when all strikes were combined (black line).

Propulsive phase

During the propulsive phase, the combined acceleration from the point of first motion until the sculpin crossed the trajectory of the sand eel was defined as the strike performance ($VeDBA_{Mean}$). $VeDBA_{Mean}$ increased with postural curvature (P=0.010, r^2= 0.386) (Fig.4b), and significantly differed between attack types, with higher $VeDBA_{Mean}$ for type B attacks (P=0.048; Fig.7.6). There was no significant correlation between $VeDBA_{Mean}$ and strike distance (P>0.443), relative position (P>0.060) or relative orientation (P>0.068).

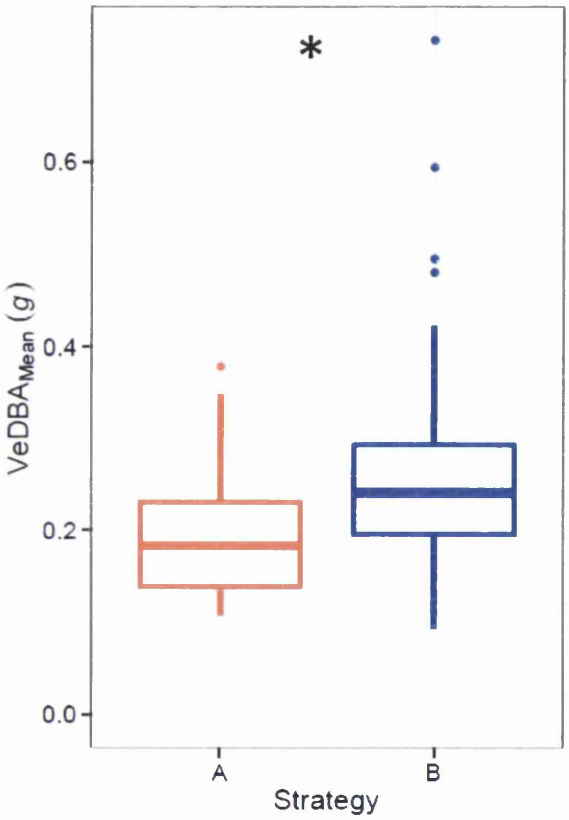


Figure 7.6. Mean acceleration ($VeDBA_{Mean}$) of fish over the duration of the attack with a bending response towards the convex (A:orange) or the concave (B:blue) side of the body. Significant differences between attack types are shown by the asterisk.

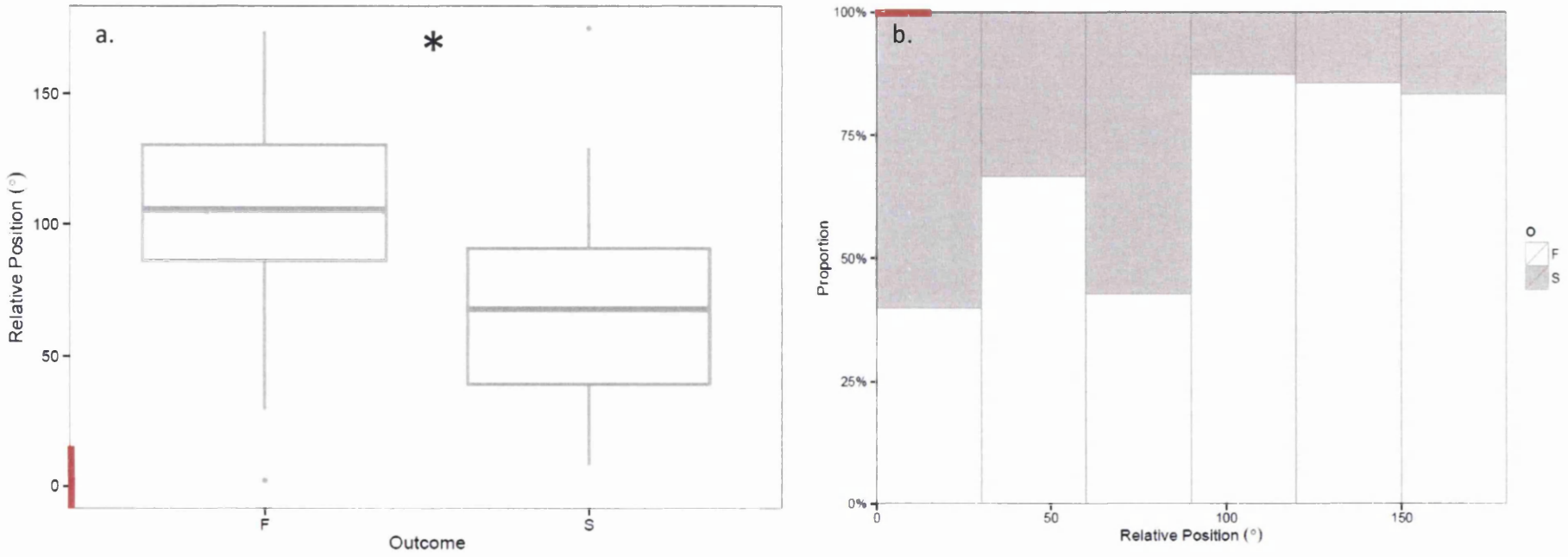


Figure 7.7. Relative position of sculpin to the sand eel in relation to the outcome of the predation attempt (a) and as a proportion of failed and successful attempts (b). The red line corresponds to the angle when the prey was directly in front of the sculpin.

When only the successful attacks were compared, strike duration (F=5.568, P=0.047) and mean acceleration (F=4.616, P=0.048) significantly differed between attack types (Fig.7.8), with faster attacks significantly more likely to be successful for type A attacks (Fig.7.8a) and higher $VeDBA_{Mean}$ for successful B attacks (Fig.7.8b).

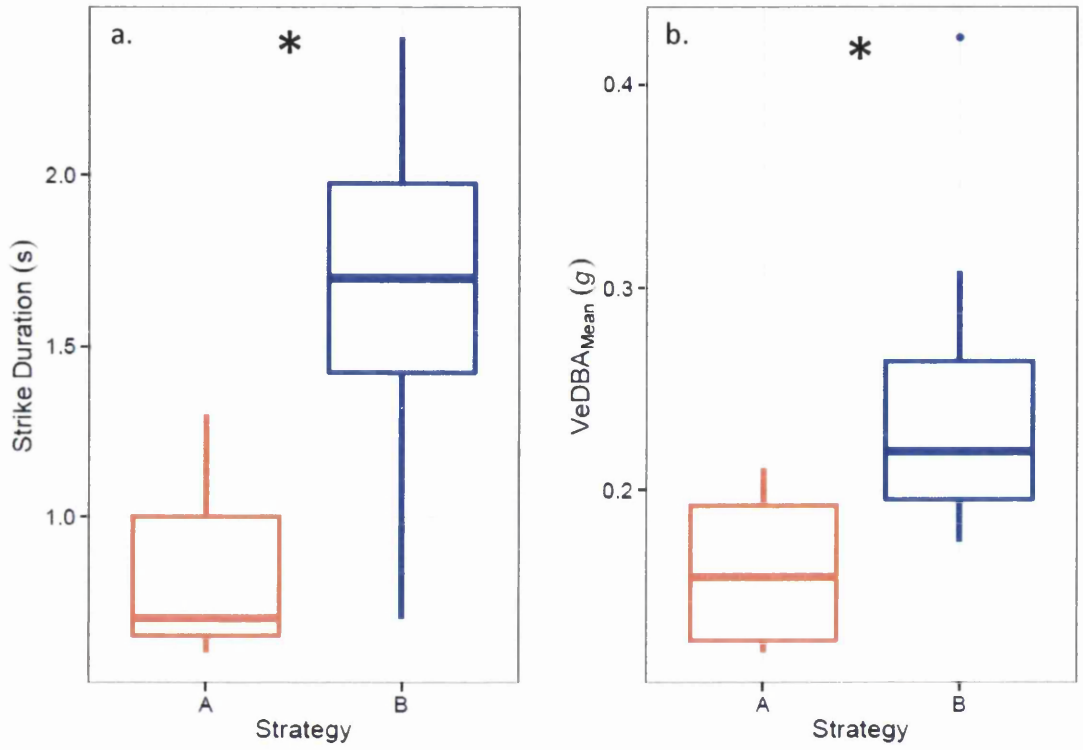


Figure 7.8. Significant difference between attack type (A = Convex; B = Concave) in relation to the strike duration (a) and mean acceleration (b) for successful strikes only.

Discussion

Predators must adapt their behaviour to maximise the likelihood of catching their prey; with various factors affecting the success likelihood of strikes, including locomotor morphology, speed and manoeuvrability. In the present experiment, the attack type and the relative position and orientation of the sculpin to the sand eel affected the success likelihood of the strike.

Sculpin visually tracked their prey in a similar manner documented for strikes by the stone fish (*Synanceia verrucosa*) (Grobecker, 1983). However, in addition to vision, sculpin may also gain positional information about the location of

their prey through the lateral line, as noted for another species of sculpin, *Cottus bairdii*, (Coombs and Conley, 1997). This positional information may enable predators to adapt the timing and distance of their strike to maximise the likelihood of success.

In addition to using sensory cues to track the location of the sand eel, sculpin also bend their caudal fin prior to the strike, resulting in either an increase (B) or decrease (A) in postural curvature. This standing S strike has also been noted for one of the two attack types initiated by tiger musky, *Esox* sp. (Webb and Skadsen, 1980) and is in contrast to the moving S strike shown for short horned sculpin, *Myoxocephalus Scorpius* (Beddow and Johnston, 1995), and pike, *Esox Lucius* (Harper and Blake, 1991). The difference between standing and moving S strikes may reflect a disassociation in strategies between species, with the standing strike providing a means to maximise the power generated (increase kinetic energy) whilst minimising detection by the prey, as noted in strikes for tiger musky (New et al., 2001).

General strike trends

Prey size can affect reaction distance (Domenici, 2002), with some studies noting an increase with prey size (Grant and Noakes, 1987; Paglianti and Domenici, 2006) and others noting a decrease (Abrahams, 1995). Furthermore, as the mouth size of a predator increases, the distance that the prey can escape decreases (Webb and Buffrénil, 1990) and thus, relatively large predators may not need to manoeuvre quickly for prey capture. For sculpin in this study, we found no significant correlation between predator size and strike distance, suggesting that other factors had a greater effect on the outcome of the strikes.

The performance of the strike was estimated from tri-axial acceleration (mean vectorial dynamic body acceleration; $VeDBA_{Mean}$) from the point of first motion until the trajectories of the predator and prey crossed. There was no significant correlation between strike performance and predator size (length or weight), reconfirming that fast-start accelerations are size independent (Domenici and Blake, 1997; Webb, 1978a).

At the point of first motion, the average relative position of the sculpin to the sand eel was 96°, which may relate to the field of view of the sculpin, with attacks initiated when the sand eel were to the side of the sculpin, and thus in direct line of sight. S-strikes were more likely to be successful when prey were located to the side or ahead of the sculpin. In addition to differences in position, the average orientation was near perpendicular (99°) mirroring results for pike (*Esox* sp.) attacking fathead minnows (*Pimephales promelas*) (Webb and Skadsen, 1980). The high incidence of strikes at this attack angle may relate to the predator aiming to keep the escape paths of the prey aligned to their strike path. Previous studies note that predators have a better chance of catching the prey when the predator-prey orientation approaches nose-to-nose orientation (180°) (Hoogland et al., 1956; Webb, 1976). However, for sit and wait predators like sculpin, attacks from the side may maximise the body surface area of prey at which to aim, as noted in Domenici and Blake, (1997). Thus, the attack orientation may be dependent on the morphology of the predator.

Comparing strikes A & B

Comparisons were made between type A and type B strikes, to assess whether there were differences in behaviour and energy use when sculpin attacked using these different methods. As postural curvature increased, strike performance ($VeDBA_{Mean}$) increased, resulting in higher acceleration for type B compared to type A attacks. Turesson et al., (2009) found a similar increase in locomotor performance with postural curvature for escaping gobies, suggesting that sculpin modulate the acceleration exerted during a strike by changing the position of their caudal fin during the preparatory phase. Thus, sculpin may be able to alter their attack strategy to reflect the behaviour of their prey as a means to maximise the effort exerted compared to the energy gained.

The higher acceleration ($VeDBA_{Mean}$) exerted during type B attacks may be the reason for the difference in success likelihood of A & B strikes, as B strikes enable sculpin to intercept prey largely independent of strike distance within a certain range. However, the increased energetic cost incurred using type B attacks may mean that when costs and benefits are weighed up, type A attacks are

energetically cheaper when low force strikes are sufficient to catch the prey, for example when prey are relatively close.

The success of type A attacks was affected by the duration of the strike, with faster strikes more likely to result in a successful attempt and an increased likelihood of failure when prey were more than 21cm from the predator. In contrast, type B attacks resulted in successful attacks independent of strike duration or prey distance. The effect of strike duration on success for type A attacks, mirrors the principle that strikes should be as quick as possible to reduce the probability of escape (Domenici, 2001; Hart, 1997). However, the lack of effect of strike duration for type B attacks may be due to the increased acceleration exerted during B strikes, resulting in successful strikes independent of distance or duration.

Conclusion

Results from this study highlight the complex interaction between attack strategy and the success likelihood of strikes, with both locomotory and non-locomotory variables affecting the outcome. Great sculpin use information about the position and orientation of their prey to aid in capture. Future studies should address the attack type for other prey species to better understand adaptability and the importance of strike behaviour and performance on predator-prey interactions both in the laboratory and field, with data logger accelerometers providing a useful tool for tracking the performance of fish.

List of symbols and abbreviations

A	strike type A; corresponding to a convex (negative curvature)
B	strike type B; corresponding to a concave (positive curvature)
K	Fulton's *K* condition factor
TL	total length (cm)
Tt	point of first motion
Tt_{-1}	point prior to first motion
VeDBA	vectorial dynamic body acceleration (g)
$VeDBA_{Mean}$	mean vectorial dynamic body acceleration (g)
W	weight (g)

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Chapter 8

Synopsis

Serena R. Wright

Biotelemetry enables us to track animals in their natural habitats or in conditions where direct observation is restricted or impossible. For example, accelerometer loggers provide information about the behaviour (Gleiss et al., 2011b; Whitney et al., 2010) and energy use (Gleiss et al., 2011a; Wilson et al., 2013) of fish in the laboratory and field. However, as accelerometers and other biotelemetry devices are increasingly used to track animals, the methods used to deploy and extract information from tags need to be tested and refined.

There are important ethical and scientific reasons why animal-attached tags need to impact their carriers minimally (Adams et al., 1998) and one aspect of this regarding implanted systems for fish that has received scant attention is the importance of using the appropriate material to encase tags prior to intraperitoneal insertion. Chapter 2 deals with this in some detail but also emphasises the time-based elements in tagging, specifically that the adoption of 'normal' behaviour is likely to be a function of time since tagging (cf. Wilson and McMahon, (2006)). There is no simple, single solution to this, and my expectation is that there will be some sort of functional response, with aberrant behaviours dropping rapidly over time, perhaps with a half-life-type expression with the specific value of the half-life being a function of the procedure and the species. The concept that the half-life idea might be used for tagged animals is perhaps important in that half-lives never allow the functional response to drop to zero and there are good reasons to believe that tag effects are similar in that tags will always have some detrimental impact on the tag carriers (Drenner et al., 2012; Methling et al., 2011; Thorstad, 2001). At its most base level though, researchers certainly need to give fish enough time to 'recover' from tagging events before the start of studies, particularly if studies are to focus on behaviour and energy use.

In addition to ensuring that robust procedures are in place at deployment and that appropriate time is given for animal habituation to the tag and procedure, the methods that we use to process tag data after retrieval are equally vital to minimise misinterpretation of data. This is particularly true of accelerometer data, which are considered complex and, for that reason are liable to cause confusion. Shepard et al., (2008) point to the criticality of understanding the various components of acceleration data and highlight that dividing the signal into the static (gravity-derived) and dynamic (animal muscular contraction-derived) components provides the basis for election of a powerful key with which to define various behaviours. Although acknowledged by various authors (e.g. Gómez Laich et al.,(2011)), the issue of how to isolate the two components has not been given proper theoretical consideration. Chapter 3 does this, defining a new procedure for processing accelerometer data to separate the static from the dynamic acceleration, and provides a robust means of measuring postural (static) changes and muscle-induced (dynamic) changes in acceleration.

The acid test of this approach will come by comparing methods used in the literature (running means (e.g. (Gleiss et al., 2013; Shepard et al., 2008)) and pass filters (e.g. (Sato et al., 2003; Watanuki et al., 2003))) to the proposition put forward in this thesis. The nub is the degree of difference between methods and what this leads us to conclude with respect to derivation of behaviours.

The importance of aerobic and anaerobic performance has been known to athletes for decades (e.g. (Kindermann et al., 1979; Marconi et al., 1985)) and its mention for fish also has a long history (Weihs, 1974). Given that the pathway used determines the length of time that the relevant behaviour can be engaged, whether a response is aerobic or anaerobic is clearly hugely significant for wild animals where, for example, protracted versus short-lived predator-prey pursuits call for different performances. In fact, it has been postulated that some of the highest power requirements exhibited by animals relate to either escape by prey or pursuit by a predator (Brose et al., 2008) and the ultimate limits to the performance, perhaps whether prey will be caught or not, will likely be determined by the length of the chase.

Among vertebrates, pelagic fish represent a somewhat different case to terrestrial vertebrates. Where prey fish are associated with underwater topography, they can hide within and between features, as can many terrestrial vertebrates and so may be well served by a single, short escape reaction – a fast start – provided by white muscle. In pelagic zones, escape may be modulated by water turbidity because if prey fish can move beyond the perception of visual predators, their escape is more likely. Thus, clear oceanic waters, for example, are likely to induce longer pursuits which may go some way to explaining the predominance of red muscle in pelagic fish.

Overall though, whether an escape from predation has occurred aerobically or anaerobically, the exercise still has to be "paid for" with oxygen and since energy has been called 'the currency of life' there are good reasons for biologists to want to quantify this. The differences in the replenishment rate of oxygen to the system during aerobic and anaerobic exercise are substantial, however, making the determination of metabolic power, and specifically when the power is being used, problematic using conventional respirometry. The work in chapters 4 & 5 shows that accelerometers have the potential to be used to estimate both aerobic and anaerobic energy use of fish in the field, importantly, demonstrating that metrics derived from accelerometers can act as a proxy for instantaneous, rather than overall summed energy use. Although aerobic energy use has already been linked to acceleration in sockeye salmon (Wilson et al., 2013) and hammerhead sharks (Gleiss et al., 2011a), no studies to date have linked acceleration to anaerobic energy use (excess post

exercise oxygen consumption; EPOC) in fish (but see Robson et al., (2012) for scallops). EPOC results from energetic costs associated with high intensity activity, such as burst-swimming, and has previously been estimated in the laboratory on a number of fish species including sockeye salmon (Lee et al., 2003) and qingbo (Zhao et al., 2012). Estimates of EPOC enable researchers to understand aerobic limits better in relation to important environmental variables such as temperature (Zeng et al., 2010) and feeding (Fu et al., 2007). The application of accelerometers in the quantification of EPOC may provide an invaluable tool for studies of fish energetics in the field, although further experiments are required to validate results. By tracking the activity-specific energy use of fish in the field, important information about migration costs and behavioural changes with time can be better understood. For example, for European eel, *Anguilla anguilla*, the ability to track the free-ranging energy use of adults on their migration to spawning grounds is a real, and exciting, new prospect, which will aid in our understanding of these enigmatic species. Data storage tags and satellite transmitting tags are already used in the study of eel migration (Westerberg et al., 2007; Westerberg et al., 2013), but accelerometers may improve our understanding by enabling us to assign a cost to these long migrations, as shown in a early study by Westerberg, (1984).

The ability to quantify the energy use of fish in the field will enable us to gain a better understanding of energy budgets in association with short and long term changes in biotic and abiotic variables, such as seasonal aggregations (Wilson et al., 2001) and changes in temperature (Fraser et al., 1993). In addition to gaining insights into energetic costs, behavioural changes are also important in the study of fish ecology. Chapters 6 and 7 provide examples of the application of accelerometers to the study of fish behaviour. As ectothermic fish experience changes in their environment it can be assumed that there will be physiological and behavioural implications (Brill, 1994; Dickson et al., 2002; Eliason et al., 2011).

Chapter 6 tracks the behavioural response of two stocks of Atlantic cod to temperature change. Changes in activity level were then linked to the amount of energy used for locomotion at different temperatures, highlighting significantly different responses of the two populations to temperature change. The link between locomotor activity and temperature shows the potential for this technology to be used to better understand the driving factors which influence spatio-temporal movements of fish. This information may in turn provide valuable information for the protection and sustainable management of fish populations, especially in situations where environmental conditions are changing (Walther et al., 2002) and the health and viability of populations are under threat (Gozlan et al., 2005).

Accelerometer tags were also used to track the strike behaviour of the sit-and-wait predator, the great sculpin (chapter 7). Postural changes in behaviour prior to the strike affected the amount of acceleration exerted, which in turn affected the outcome. The high resolution information provided by the tags show that accelerometers can be used as an effective tool for quantifying high resolution behaviours such as "fast-starts" (Broell et al., 2013) which may enable us to identify specific behaviours both in the laboratory and field without the need for direct observation. This information may then be used to gain a better understanding of behaviours of fish in the field, with the potential to accurately define areas of importance for events such as feeding and spawning, as shown in a recent study by Gleiss et al., (2013).

Though accelerometer data storage tags have the capacity to provide previously unattainable information about the estimated energy use and behaviour of fish in the field, accelerometers cannot be used to track the energy use and behaviour of immobile animals. For example they do not provide direct costs associated with bodily functions induced by changes in temperature (Claireaux and Lagarde, 1999; Hein and Keirsted, 2012) , reproduction (Lambert and Dutil, 2000) and digestion (Dupont-Prinet et al., 2009; Fitzgibbon et al., 2007). Additionally, limits to the memory capacity and battery life of tags reduce their applicability for long term field studies; though advances in technology are providing tags that last longer and can store ever more information.

Accelerometers have long been used to classify the behaviour of a huge range of animals, including humans (Foerster et al., 1999), cats (Watanabe et al., 2005), pigs (Cornou and Lundbye-Christensen, 2008), cormorants (Gómez Laich et al., 2011), geese (Hawkes et al., 2014) and whale sharks (Gleiss et al., 2013). The classification of activities requires analysis to decode this data from the accelerometers, including visually comparing data to video footage (Yoda et al., 2001) and classifying activities based on mathematical transformations (Cornou and Lundbye-Christensen, 2008; Watanabe et al., 2005). Video footage analysis is time-consuming, subjective and requires high visibility, whilst mathematical transformations involve complex mathematical processing, which can limit wide spread application for behavioural researchers. Difficulties associated with methods to process raw acceleration may be overcome by using automated analysis methods

as used in the derivation of human activities (Burchfield et al., 2007; Bussmann et al., 1998). An automated and simple analysis system for tracking the behaviour of fish in the field would be widely applicable and would make accelerometry more accessible as a tool to an ever broader audience.

As electronic tags become smaller with higher sensitivities and larger data storage capacities, new prospects arise in the study of fish behaviour. With improvements in our understanding of methods to process acceleration and to extract useful information we will be able to gain ever more information about animals in the field. This information is not only useful for researchers wishing to better understand reasons for animal movements; it may also serve as a tool to underpin policy to maintain healthy ecosystems and to ensure that populations are protected from over-exploitation.

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Appendices

Appendix 1: Supplementary information for Chapter 3

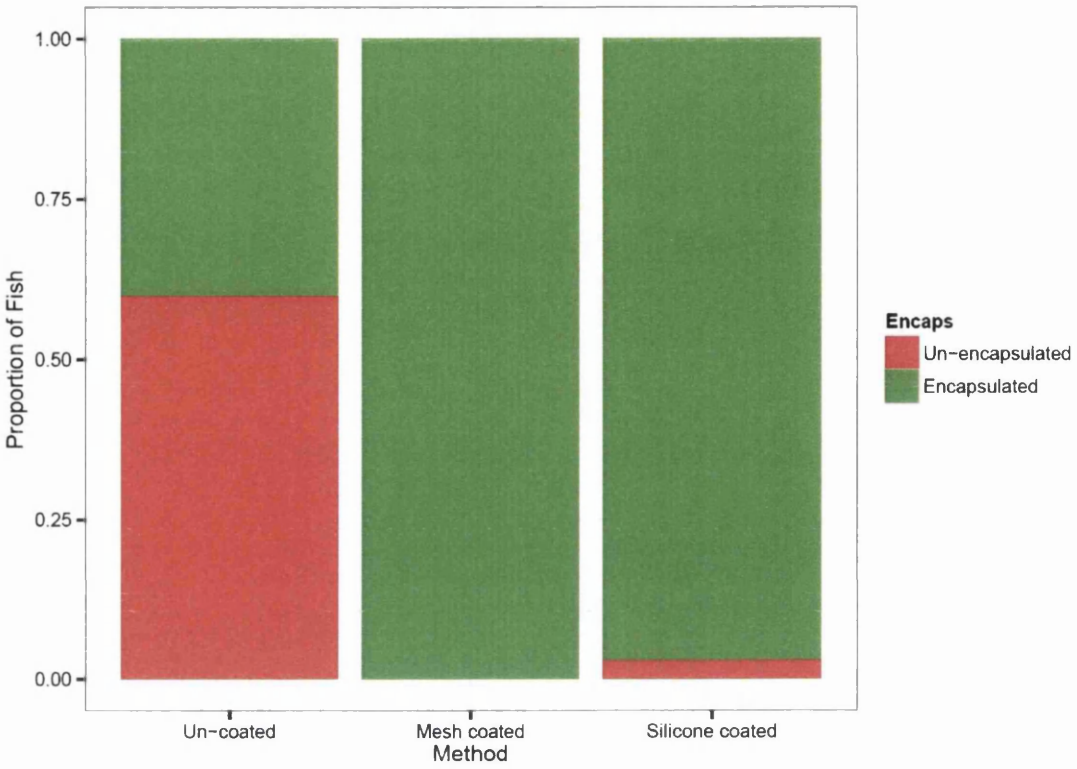

Figure S2.1. Tag attachment method in relation to whether tags were encapsulated by the end of the experiment.

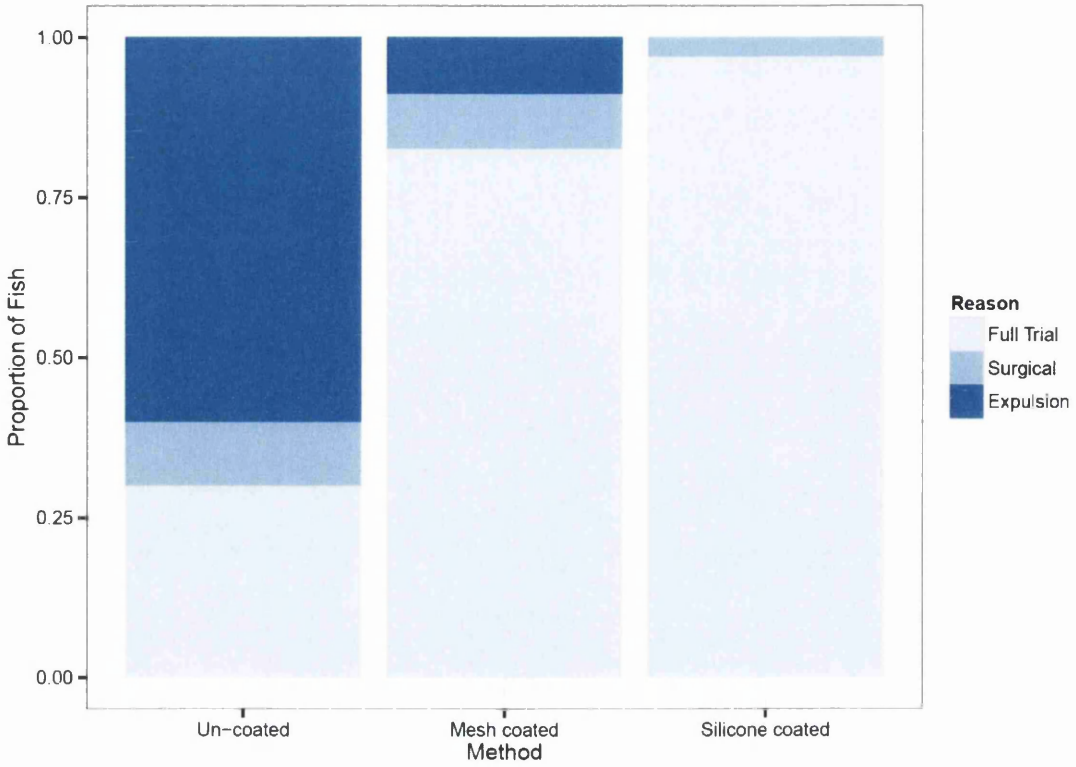

Figure S2.2. Tag attachment method in relation to cause of death (full trial, surgical reason, expulsion of tag).

Appendix 2: Supplementary information for Chapter 3

Calculating the optimal smoothing algorithm R Script

Below is the R script used in order to calculate the optimal smoothing algorithm from the sway axis of tri-axial acceleration data, and may be copied and pasted into R's own script editor. Two files should be created prior to running the R-script; (1) "Fish_Lengths.csv" containing two columns with information about the ID and length of the fish, (2) a section of steadily swimming sway acceleration should be extracted from a fish dataset, ensuring that clear peaks and troughs corresponding to the tail beat of the fish are present. These files should be located in the following subdirectory: "G:\\Acceleration_Experiment\\Bass\\10Hz\\"

with the fish type (Bass) and the sampling frequency (10Hz) as subfolders.

'File_Location' should indicate the directory containing the sway acceleration file and the Fish_Lengths.csv file.

```
File_Location <- "G:\\Smoothing_Files\\"
List_of_Species <- c("Bass","Cod","Trout","Eel","BTRS","Sculpin","Wha","Nur") # Name the
species involved in the model
COI <- 15 # column of interest representing the column within the data file which will be
used to predict the optimal smoothing window
Crit_Prop <- 0.9 # the critical proportion of the power spectrum attributed to the static
acceleration alone (from 0 to 1)
Sampling_Win <- 60 # define the sampling window for replicates in seconds

Spec_N_Prop(List_of_Species, COI, Crit_Prop, Sampling_Win)

Spec_N_Prop <- function(List_of_Species, COI, Crit_Prop, Sampling_Win){

# Automatically attach the required packages

  ##############################################################################
  ##############################################################################
  required.packages <- c("pastecs", "psych", "silvermantest","fts")
  new.packages <- required.packages[!(required.packages %in%
installed.packages()[,"Package"])]
  if(length(new.packages)>=1){lapply(new.packages, install.packages)}
  lapply(required.packages, require, character.only=T)
  ##############################################################################
  ##############################################################################


  # Read in the data files
  Species_list <- List_of_Species
  Opt_Spec_Hz <- data.frame(matrix(ncol=5,nrow=100))
  Counta <- 1


  for(New_Sp in unique(Species_list)){

    #New_Sp <- unique(Species_list)[8]

    Sp <- New_Sp # Species of interest
    Hz_Folders <- list.files(paste(File_Location,Sp,sep=""))

    for(j in unique(Hz_Folders)){
```

```
      #j <- Hz_Folders[1]
      Hz_Folder <- j

      a <- list.files(paste(File_Location,Sp,"\\",Hz_Folder,sep=""))
      Hz <- as.numeric(substring(Hz_Folder,4,nchar(Hz_Folder)))

      # Read in the length and weight data
      LW <- read.csv(paste(File_Location, "Fish_Lengths.csv",sep=""))

      Samp <- seq(0.2,10,0.2); Samp <- (Samp * Hz)+1 # Show sampling window length based on
Hz

      # Create empty dataframe for stats
      C_N <- c("ID","Samp","Hz","Group","Prop_Max")


#pdf(file=paste("E:\\Acc_Methods\\Static_Acceleration\\Figures\\Spectral\\",New_Sp,".pdf",sep
=""),
      #    width=8.27,height=5.83,paper='special')

      # Split by species
      for(i in unique(a)){
        #i <- a[1]

        df <- read.csv(paste(File_Location,Sp,"\\",Hz_Folder,"\\",i,sep="")) # Read in the
datafile
        df$Static <- as.numeric(sqrt((df$FX^2)+(df$FY^2)+(df$FZ^2)))
        df$R_Time <- strptime(df$FT, "%d/%m/%Y %H:%M:%OS")
        #  plot(density(na.omit(df$Static)))
        Den <- density(na.omit(df$Static))
        Mx_Den <- which(Den$y == max(Den$y))
        Grav <- Den$x[Mx_Den]
        #  abline(v=Grav,col="red",lty="dashed")


        # Smooth over 10 future and 10 past samples
        f19 <- c(1/10,2/10,3/10,4/10,5/10,6/10,7/10,8/10,9/10,1,9/10,8/10,7/10,6/10,
                 5/10,4/10,3/10,2/10,1/10)
        Data_sm <- as.numeric(filter(df$FZ, f19, sides=2))
        #plot(df$FZ[1:1000],type="l")
        #lines(Data_sm[1:1000],col="red")

        # Calculate the local mean and standard deviation
        library("caTools")
        Local_m<- runmean(x=Data_sm, k=100)
        Local_sd<- runsd(x=Data_sm, k=100)

        Autolevel <- (Data_sm - Local_m)/Local_sd


        peaks<-function(series,span=20){
          z <- embed(series, span)
          s <- span%/%2
          v<- max.col(z) == 1 + s
          result <- c(rep(FALSE,s),v)
          result <- result[1:(length(result)-s)]
          result
        }
        p=which(peaks(Autolevel))

        df$Beat <- "F"
        df$Beat[p] <- "T"

        # Calculate TBF (Hz = number per second)
        Elapsed_T <- df$R_Time[nrow(df)] - df$R_Time[1]
        Time <- Elapsed_T[[1]]*60
        TBF_Hz <- length(which(df$Beat == "T"))/Time

        # Create grouping column
        # Calculate ODBA and static acceleration over 30 second stretches of acceleration
        Rw <- Hz*Sampling_Win;    Rws <- seq(1,nrow(df),Rw)
        if(Sp == "Sculpin"){ df$Group = paste("G",1,sep="")} else {
          df$Group <- NA
```

```
          for(c in 1:(length(Rws)-1)){
            ca <- Rws[c]; cb <- Rws[c+1]
            df$Group[ca:cb] <- paste("G",c,sep="")
          }
        }

        df_A <- df
        df_A <-  remove.na.rows(df_A)

        for(Grp_Fun in na.omit(unique(df_A$Group))){

          Grp_Fun <- df_A$Group[1]
          df <- df_A[df_A$Group == Grp_Fun,]

          All_Smx <- data.frame(matrix(ncol = length(unique(Samp)), nrow = nrow(df)))
          All_Dyx <- data.frame(matrix(ncol = length(unique(Samp)), nrow = nrow(df)))
          count2 <- 1


          Statistics <- data.frame(matrix(ncol = 34, nrow = 0))
          colnames(Statistics) <-
c("ID","Samp","Hz","Group","ODBA_Mn","ODBA_Med","ODBA_SD","ODBA_N","ODBA_SE","ODBA_Mx",
"ODBA_Range","ODBA_Vari","ODBA_Coef.Var","ODBA_Skew","ODBA_kurtosis","Sway_Mod","Pos",
"Neg","St_Mn","St_Med","St_SD","St_N","St_SE","St_Mx","St_Range","St_Vari","St_Cv_All",
"St_Cv_x","St_Cv_y","St_Cv_z","St_Skew","St_kurtosis","St_Mode","Grav")

          for(b in unique(Samp)){
            N <- rep(1/b,b) # Use the different sampling windows set the filter
            df$Smx <- filter(df$FX, N, method = "convolution",sides=2) # filter axis x
            df$Smy <- filter(df$FY, N, method = "convolution", sides=2) # filter axis y
            df$Smz <- filter(df$FZ, N, method = "convolution", sides=2) # filter axis z
            df$Smx <- as.numeric(df$Smx); df$Smy <- as.numeric(df$Smy); df$Smz <-
as.numeric(df$Smz)

            df$Raw_Dyx <-df$FX-df$Smx ; df$Raw_Dyy <-df$FY-df$Smy;  df$Raw_Dyz <-df$FZ-df$Smz
            #df$Dyx <- abs(df$FX-df$Smx); df$Dyy <- abs(df$FY-df$Smy); df$Dyz <- abs(df$FZ-
df$Smz)# extract the dynamic component
            df$Dyx <- df$FX-df$Smx; df$Dyy <- df$FY-df$Smy; df$Dyz <- df$FZ-df$Smz# extract
the dynamic component
            df$ODBA <- as.numeric(sqrt((df$Dyx^2)+(df$Dyy^2)+(df$Dyz^2)))
            df$Static_All <- as.numeric(sqrt((df$Smz^2)+(df$Smx^2)+(df$Smy^2)))
            df$Static_x <- as.numeric(sqrt((df$Smx^2))); df$Static_y <-
as.numeric(sqrt((df$Smy^2)))
            df$Static_z <- as.numeric(sqrt((df$Smz^2)))
            df$Pos <- NA; df$Pos[which(df$Raw_Dyz > 0)] <- "Pos";
            df$Neg <- NA; df$Neg[which(df$Raw_Dyz < 0)] <- "Neg"
            #    lines(as.numeric(df$Static),col="red",lwd=count)

            Spect <- spec.ar(na.omit(df$Smy), log="no",plot=F)

            # VeDBA (as ODBA)
            ODBA_Mn<-with(df, aggregate(list(ODBA=Dyz), by=list(Group=Group),FUN=mean,
na.rm=T))
            ODBA_Med <- with(df,aggregate(list(ODBA=Dyz), by=list(Group=Group),FUN=median,
na.rm=T))
            ODBA_SD<-with(df, aggregate(list(ODBA=Dyz), by=list(Group=Group),FUN=sd,
na.rm=T))
            ODBA_N<-with(df, aggregate(list(ODBA=(!is.na(Dyz))), by=list(Group=Group),
FUN=sum, na.rm=T))
            ODBA_SE<-ODBA_SD[,2]/sqrt(ODBA_N[,2])
            ODBA_Mx<-with(df, aggregate(list(ODBA=Dyz), by=list(Group=Group),FUN=max,
na.rm=T))
            Range <- rep(stat.desc(df$Dyz)[6],nrow(ODBA_Mn[1]))
            Var <- rep(stat.desc(df$Dyz)[12],nrow(ODBA_Mn[1]))
            Coef.Var <- rep(stat.desc(df$Dyz)[14],nrow(ODBA_Mn[1]))
            ODBA_Skew <- rep(describe(df$Dyz)[11],nrow(ODBA_Mn[1]))
            ODBA_Kurt <- rep(describe(df$Dyz)[12],nrow(ODBA_Mn[1]))
            Den_Z <- density(na.omit(df$FZ))
            Sway_Mode <- rep(Den_Z$x[which(Den_Z$y == max(Den_Z$y))],nrow(ODBA_Mn[1]))
            Pos <- with(df, aggregate(list(Pos=(!is.na(Pos))), by=list(Group=Group),FUN=sum,
na.rm=T))
            Neg<- with(df, aggregate(list(Neg=(!is.na(Neg))), by=list(Group=Group),FUN=sum,
na.rm=T))
```

```
            ODBA_Stats <- cbind(i,b,(b-
1)/Hz,ODBA_Mn,ODBA_Med[,2],ODBA_SD[,2],ODBA_N[,2],ODBA_SE,ODBA_Mx[,2],

Range,Var,Coef.Var,ODBA_Skew[1],ODBA_Kurt[1],Sway_Mode[1],Pos[,2],Neg[,2])
            # Smoothed
            St_Mn<-with(df, aggregate(list(Static=Smz), by=list(Group=Group),FUN=mean,
na.rm=T))
            St_Med<-with(df, aggregate(list(Static=Smz), by=list(Group=Group),FUN=median,
na.rm=T))
            St_SD<-with(df, aggregate(list(Static=Smz), by=list(Group=Group),FUN=sd,
na.rm=T))
            St_N<-with(df, aggregate(list(Static=(!is.na(Smz))), by=list(Group=Group),
FUN=sum, na.rm=T))
            St_SE<-St_SD[,2]/sqrt(St_N[,2])
            St_Mx<-with(df, aggregate(list(Static=Smz), by=list(Group=Group),FUN=max,
na.rm=T))
            St_Range <- rep(stat.desc(df$Smz)[6],nrow(St_Mn[1]))
            St_Var <- rep(stat.desc(df$Smz)[12],nrow(St_Mn[1]))
            St_Cv_All <- rep(stat.desc(df$Static_All)[14],nrow(St_Mn[1]))
            St_Cv_x <- rep(stat.desc(df$Static_x)[14],nrow(St_Mn[1]))
            St_Cv_y <- rep(stat.desc(df$Static_y)[14],nrow(St_Mn[1]))
            St_Cv_z <- rep(stat.desc(df$Static_z)[14],nrow(St_Mn[1]))
            St_Skew <- rep(describe(df$Smz)[11],nrow(St_Mn[1]))
            St_Kurt <- rep(describe(df$Smz)[12],nrow(St_Mn[1]))
            St_Mode <- rep(density(na.omit(df$Smz))$x[which(density(na.omit(df$Smz))$y ==
max(density(na.omit(df$Smz))$y))],nrow(St_Mn[1]))
            St_Stats <- cbind(St_Mn,St_Med[,2],St_SD[,2],St_N[,2],St_SE,St_Mx[,2],

St_Range,St_Var,St_Cv_All,St_Cv_x,St_Cv_y,St_Cv_z,St_Skew[1],St_Kurt[1],St_Mode[1])

            Stats <- cbind(ODBA_Stats,St_Stats[,2:ncol(St_Stats)],Grav)
            colnames(Stats) <- colnames(Statistics) # relabel so that the column names match
            Statistics <- rbind(Statistics,Stats)

            # Add new smoothed term to all dataframe
            All_Smx[count2] <- df$Smz
            All_Dyx[count2] <- df$Raw_Dyz
            count2 <- count2 + 1
          }
          Statistics <- Statistics[-1,]
          Statistics$Propo <- NA
          Statistics$Pro <- NA


##############################################################################
          # Proportion calculations

          Spect <- spec.ar(na.omit(df$Smz), log="no",plot=F)


          peaks <- function (x, thresh = 0)  {
            pks <- which(diff(sign(diff(x, na.pad = FALSE)), na.pad = FALSE) < 0) + 2
            if (!missing(thresh)) {
              pks[x[pks - 1] - x[pks] > thresh]
            }
            else pks
          }

          All_Spec <- data.frame(matrix(ncol=length(unique(Samp)),nrow=length(Spect$spec)))
          All_Prop <- data.frame(matrix(ncol = 3, nrow = ncol(All_Smx)))

          for(pp in 1:(ncol(All_Smx))){
            Smx_1 <- na.omit(All_Smx[,pp])
            Dyx_1 <- na.omit(All_Dyx[,pp])
            if(length(Smx_1) > 1) {
              Spec_1 <- spec.ar(na.omit(All_Smx[,pp]),plot=F)
              All_Spec[,pp] <- Spec_1$spec

              # Designate proportion of signal to each frequency
              Total <- sum(Spec_1$spec) # total noise on all frequencies
              Freq_Prop <- Spec_1$spec/Total
              Spectrum <- Spec_1$spec
              Highest_F <- max(Freq_Prop) # Max
```

```
            Pro <- sum(Freq_Prop[which(Freq_Prop == Highest_F)-50:which(Freq_Prop ==
Highest_F)+50])
            N_Mod <- nr.modes(All_Smx[,pp])
            S <- c(0,Spectrum)
            pk <- (peaks(S,thresh=0.1))
            if(length(pk) == 0){pk <- peaks(S,thresh=(max(S)*0.0005))}

            All_Prop[pp,1] <- Statistics$Hz[pp]
            All_Prop[pp,2] <- Pro
            All_Prop[pp,3] <- NA
            if(length(pk) >= 1) {All_Prop[pp,3] <- length(pk)}

          }
          if(length(Smx_1) == 0){
            All_Spec[,pp] <- NA
          }
        }

        Statistics$Propo <- All_Prop[1:nrow(Statistics),3]
        Statistics$Pro <- All_Prop[1:nrow(Statistics),2]

        Stat <- Statistics

        hh <-COI
        Select <- Stat[,hh]


        ######################################################################
        Opt_P <- Stat$Hz[which(Select == min(Select))]
        Opt_PR <- which(Select == min(Select))
        Min_Prop <- which(All_Prop[Opt_PR:nrow(All_Prop),2] >= Crit_Prop) + (Opt_PR-1)

        if(length(Min_Prop) >= 1){
          Eg_Op_N <- min(Min_Prop)
          Opt_P <- Stat$Hz[min(Min_Prop)]
          Opt_PR <- min(Min_Prop)
        } else {
          Eg_Op_N <- min(Opt_PR)
          Opt_P <- Stat$Hz[Opt_PR]
        }


        b <- (Opt_P*Hz)+1 ; N <- rep(1/b,b) # Use the different sampling windows set the
filter
        Eg_Op <- filter(All_Smx[,Opt_PR], N, method = "convolution",sides=2); Eg_Op <-
as.numeric(Eg_Op)

        # Split the window in to 1 large box with two smaller rows
        plot_order <- matrix(c(1,1,1,1,2,3),nrow=2)
        mylayout <- layout(mat=plot_order)

        plot(y=na.omit(df$FZ),x=na.omit(as.numeric(rownames(df))/Hz),type="l",xlab="Time
(s)",ylab="Acceleration (g)",
             main=paste(i,Grp_Fun,sep="_"))
        lines(x=as.numeric(rownames(df))/Hz,y=All_Smx[,Opt_PR],col="#FF6600",lwd=2)

        Spect_Op <- spec.ar(na.omit(Eg_Op), log="no",plot=F)

plot(Spect_Op$freq,Spect_Op$spec,col="#FF6600",lwd=2,xlab="Frequency",ylab="Spectrum",
             xlim=c(-0.02,0.2),type="l")

        plot(Stat$Hz,Stat[,COI],pch=21,bg="white",xlab="Smoothing Frequency (Hz)",
             ylab="Dynamic Acceleration Kurtosis")
        points(Stat$Hz[Eg_Op_N],Stat[Eg_Op_N,COI],pch=21,bg="#FF6600",col="#FF6600")

        # Calculate TBF (Hz = number per second)
        Elapsed_T <- df$R_Time[nrow(df)] - df$R_Time[1]
        Time_Format <- substring(format(Elapsed_T),(nchar(format(Elapsed_T))-
3),nchar(format(Elapsed_T)))
        if(Time_Format == "secs"){Time <- Elapsed_T[[1]]} else
          {Time <- Elapsed_T[[1]]*60 }
        TBF_Hz <- length(which(df$Beat == "T"))/Time

        Opt_Spec_Hz[Counta,1] <- New_Sp
```

```
          Opt_Spec_Hz[Counta,2] <- i
          Opt_Spec_Hz[Counta,3] <- Grp_Fun
          Opt_Spec_Hz[Counta,4] <- Opt_P
          Opt_Spec_Hz[Counta,5] <- TBF_Hz

          Counta <- Counta+1
        }
      }
      #dev.off()
      write.csv(Opt_Spec_Hz,paste(File_Location,New_Sp,i,".csv",sep=""))
    }
  }
  # Opt_Spec output
  write.csv(Opt_Spec_Hz, paste(File_Location,New_Sp,i,"_All.csv",sep=""))
}
```