Exploring the relationship between physiological performance and reproductive investment in wild fish using heart rate biologgers

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Acronyms

EMG	Electromyogram
f_{H}	Heart rate
$\mathrm{MO_2}$	Oxygen consumption rate
R%f_{H}	Percentage of routine heart rate within scope for heart rate
$\dot{\mathrm{V}}\mathrm{b}$	Cardiac output
Vs	Stroke volume

Abstract

Animals adopt various reproductive strategies to optimize their reproductive fitness. Given that reproductive behaviours typically involve high-energy activities, and the cardiovascular system distributes oxygen and nutrients that are essential for energy synthesis, scientists have hypothesized that reproductive fitness is related to an organism's physiological performance (i.e., cardiac function). However, limited research has tested this hypothesis. The present thesis aimed to explore the potential relationship between physiological performance and reproductive fitness by relating heart rate to parental care behaviour in smallmouth bass (*Micropterus dolomieu*), and dominance behaviour in spawning sockeye salmon (*Oncorhynchus nerka*). Male smallmouth bass parental care involves aerating the eggs to prevent suffocation, and guarding the offspring from predators and conspecifics. Alternatively, sockeye salmon spawn in high densities, and compete for a territory and a mate to optimize spawning success. It was predicted that individuals that routinely function low within their scope for heart rate would be more capable of increasing their heart rate to meet the metabolic demands of enhanced reproductive behaviours, would use less energy, and would therefore likely be more energy efficient. In contrast, individuals that routinely operate high within their scope for heart rate would be less capable of meeting the metabolic demands of reproductive behaviours, and would exhaust their energy reserves, be more vulnerable to conspecific and predator burdens, and abandon reproduction behaviours prematurely. The results in the present thesis did not detect a relationship between overall heart rate and reproductive behaviour in either species. Instead, heart rate was found to be strongly affected by the environment, and followed a diel pattern irrespective of behaviour. It is therefore proposed that reproductive behaviour is not mediated by heart rate alone, but by the combined effects of intrinsic (e.g hormones, energy density) and extrinsic (e.g. temperature, photoperiod, predator burden) factors. Hence, the present thesis demonstrates the complexity of the relationship between cardiac performance and reproductive behaviours in wild fish. Future studies are required to explore the relationship between physiological performance and reproductive fitness to better understand mechanisms driving animal life history and evolutionary ecology in changing environments.

CHAPTER 1: General Introduction

Reproductive strategies

Animals from various taxa use different reproductive strategies to optimize their reproductive fitness (Dominey 1984; Shuster 2010). These strategies are strongly influenced by the stability and predictability of the environment and resource availability, which help shape evolved breeding patterns (i.e. continuous, opportunistic, or seasonal) and mating systems (e.g. polygyny, polyandry, promiscuity, etc.) (Emlen and Oring 1977; Clutton-Brock 1989; Hau 2001; Madelaire and Gomes 2016). The evolved reproductive strategy includes behaviours that occur before and/or after fertilization, such as dominance and parental care. Dominance is attained when an individual has a characteristic or resource that places the individual at a reproductive advantage over its conspecifics (Huntingford and Turner 1987; Reeve et al. 1998). This strategy has been recorded in various taxa, and can be achieved by different methods, such as pheromone secretion (e.g. the queen ant in an ant colony, *Formicidae.*; Keller and Nonacs 1993), physical combat (e.g. dominant male lion in a pride, *Panthera leo*; Barthold et al. 2016), or female/male mate choice (e.g. plumage in male blue grosbeaks, *Passerina caerulea*; Bellentine and Hill 2003). Similarly, parental care has been observed in various species including birds, mammals, reptiles, and fish (reviewed in Clutton-Brock 1991). This is when an individual invests time and energy to feed and protect the offspring from predation (Blumer 1979). Ultimately, the selected reproductive strategy (or combination of strategies) depends on the habitat and social structure of the species, where in some cases strategies vary within a species and population (i.e. alternative mating strategies) (Gross 1996).

Teleosts (bony fish) are a group of taxa that show a diversity of reproduction strategies between and within species (Blumer 1979). For instance, twenty percent of teleost species have developed parental care strategies to optimise their offspring survival potential, where strategies range from guarders, non-guarders, and bearers (Balon 1975; Blumer 1979; see Table 1.1 for descriptions of each strategy). Alternative mating strategies within a species are also observed during competition for a mate (especially during group spawning), where some individuals are dominant while others are subordinate (Taborsky 1994; Hutchings and Myers 1994; Gross 1996; Esteve 2005). Unlike dominant individuals, subordinate individuals do not have a territory or a mate. Instead, subordinate individuals are those that reproduce by either sneaking a reproduction opportunity between a dominant pair by rapidly entering and exiting the nest, or by taking on the morphology of the opposite sex to approach a nest with lower risk of being chased (Gross and Charnov 1980; Kodric-Brown 1986). Individuals practicing such subordinate strategies are known as sneakers and satellites, respectively. Competition behaviours may vary as the reproduction period progresses, where, for instance, an individual may start dominant then take on a subordinate role over time.

For each of the aforementioned strategies, individuals experience growth, development of secondary sexual characteristics, gonad development, and behavioural displays, all of which are highly energy demanding (Roff 1983; Hendry & Berg 1999; Schreck et al. 2001). Generally, the reproductive strategy that a species evolved depends on the resulting payoff between success versus the necessary time, energy requirement, and vulnerability to predation (Magnhagen 1991; Smith and Wootton 1995). Therefore, understanding the mechanisms driving reproductive strategies of a species and the reproductive success of an individual is important because reproductive fitness is often an underlying cause behind previous behaviour decisions during earlier life history stages (survival being another cause) (Gadgil and Bossert 1970; Partridge and Harvey 1988). Describing the relationship between physiological performance and reproductive behaviour and fitness would provide insight on the range of individual variation within studied populations, animal life-history (leading up to and including reproduction and parental care performance), and ultimately, the evolutionary ecology of populations in changing environments (chapter 3 Spicer and Gaston 2009).

Hypothesis of physiological performance relating to reproductive behaviour

During reproduction life history stages, an animal's opportunity to feed is often restricted, or in some instances the animal fasts (Hinch and Collins 1991; Ridgway and Shuter 1994). Therefore, opportunities to replenish energy stores are limited or non-existent. This has driven researchers to hypothesize that physiological performance is related to reproduction behaviour and hence reproductive fitness (Ricklefs and Wikelski 2002; Spicer and Gaston 2009; Brownscombe et al. 2017), where diversity in physiological performance is the variation of physiological characteristics between individuals (Prosser 1954; chapter 1 Spicer and Gaston 2009). Specifically, it is hypothesized that individuals with poor physiological performance would be less dominant, less capable to cope with the pressures required to maintain necessary parental care behaviours, or less capable to compete for a reproductive opportunity than an individual that is more physiologically fit. This would ultimately cause the individual to have fewer offspring and a lower overall reproductive fitness.

Many studies have focused on relating morphological parameters to reproductive behaviours and fitness (Järvi 1990; Reynolds and Gross 1992; Kitano et al. 2007), however studies investigating the relationship between reproductive behaviour and physiological performance is comparatively scarce. This is likely due to the limited ability to collect real-time, continuous physiological parameters in wild, free-roaming fish (Costa and Sinervo 2004). Instead, studies relating physiological performance to reproduction behaviour and fitness have focused on periodic sampling (e.g. blood sample before and after the event) to obtain snapshots of the animal's physiological condition and to observe net changes (e.g. Iwama et al. 1989; Patterson et al. 2004; Crossin et al. 2008; Kuerthy et al. 2016). However, with recent technological development, the ability to monitor continuous, real-time data is improving and opening the opportunity to validate the hypothesis that reproduction behaviour and fitness are related to an individual's physiological performance (reviewed in Cooke et al. 2004; Cooke et al. 2016).

The cardiovascular system as mean of assessing physiological performance

An individual's physiological performance can be assessed by monitoring the activity of the cardiovascular system (Eliason and Farrell 2016). Teleosts have a two-chambered heart, where blood enters via the sinus venosus, passes through the atrium, followed by the atrioventricular canal, ventricle, and exits via the bulbus arteriosus (Icardo 2017). The cardiovascular system is responsible for distributing oxygen and essential nutrients to the muscles and organs, and for filtering waste, all of which are essential for energy synthesis (ATP) and bodily functions (Farrell et al. 2009; Eliason et al. 2013; Farrell and Smith 2017). Cardiovascular performance can be evaluated by measuring the cardiac output ($\dot{V}b$, mL min^{-1} kg^{-1}), which can be estimated according to the equation:

$$\dot{V}b = Vs \times f_H$$

where Vs is the stroke volume (the amount of blood leaving the cardiac chamber per contraction, mL beat^{-1} km^{-1}) and f_H is heart rate (the frequency of cardiac contractions, beats min^{-1})

(Thorarensen et al., 1996; Priede and Tytler, 1997). In fish, Vs depends on the ventricular filling time (Farrell 1984). Vs is driven by the combination of the aspiration force (force from the front) associated with the ventricle contractility driving venous blood into the heart, as well as the arterial contractility and the venous blood pressure (force from behind) forcing the blood out of the heart (Farrell and Smith 2017). Hence, the more the heart expands the more blood volume can enter the heart, and with a greater central venous blood pressure, Vs will increase. In contrast, the intrinsic rate of the heart is determined by pacemaker cells and is driven by the balance between adrenergic and cholinergic tone (Farrell 1993; Vornanen 2017; Farrell and Smith 2017). The cardiac pacemaker cells contain cholinergic and adrenergic nerve endings, where adrenergic innervation occurs when epinephrine stimulates the ß-adrenoceptors on the heart, which in turn activate channels permitting the cycling of intracellular calcium that stimulate the nerve (Farrell 1993; Vornanen 2017). Hence, a higher epinephrine concentration results in an increased f_H and force of contraction (i.e., elevated adrenergic tone). However, if acetylcholine molecules bind to the muscarinic receptors on the pacemaker cells, the β-adrenergic cascade is antagonized, reducing the parasympathetic drive of the pacemaker cells, and therefore the f_H decreases (i.e., elevated cholinergic tone) (Altimiras et al. 1997; Vornanen 2017).

The performance of the cardiovascular system is hypothesized to be related to reproductive performance because reproduction is energy demanding, and the cardiovascular system is linked to the oxygen transport cascade, and thus energy expenditure (Pörtner and Farrell 2008; discussed in Eliason and Farrell 2016; Harter and Brauner 2017). Hence, information on cardiac output can be further used to assess metabolic performance by estimating metabolic oxygen consumption according to the Fick equation:

$$\dot{M}O_2 = \dot{V}b \times (CaO_2 - CvO_2)$$

where $\dot{M}O_2$ is the metabolic oxygen consumption (mg O_2 kg^{-1} min^{-1}), and CaO_2 - CvO_2 is the rate of oxygen extraction from the tissues (i.e., the difference between the venous oxygen content (CvO_2) and the arterial oxygen content (CaO_2), mL dl^{-1}) (Eliason et al. 2013; Farrell and Smith 2017). It was previously thought that f_H was ineffective for estimating metabolic performance in fish because of their ability to regulate $\dot{V}b$ by altering Vs independently of f_H (Farrell 1996; Thorarensen et al. 1996). However, researchers such as Schreer et al. (2001) and Eliason et al. (2008) have demonstrated that f_H is correlated to metabolic rate, and serves an important role in regulating $\dot{V}b$ (Cooke et al. 2003a). These studies are now among many studies supporting the notion that f_H is an effective proxy for estimating metabolic performance (discussed in Cooke et al. 2016).

Given the correlation between f_H and metabolic rate, scientists have monitored f_H to investigate the energetic costs of feeding (Lucas et al. 1991; Donaldson et al. 2010), locomotion (Clark et al. 2010), predator prey interactions (Cooke et al. 2003b), fisheries interactions (i.e., release or escapement after commercial bycatch (Raby et al. 2015) or recreational catch-and-release (Cooke et al. 2003a; Prystay et al. 2017), and temperature stress (Eliason et al. 2011; Clark et al. 2011) in fish.

Introduction to thesis purpose, hypothesis, and study systems

The present thesis aimed to investigate the hypothesis that reproductive behaviour and fitness are related to an individual's physiological performance. Since reproductive behaviours are energy demanding and feeding opportunities during reproduction are often limited (or in some cases feeding ceases), and that oxygen required to create ATP is supported by the cardiovascular system, it is hypothesized that reproductive behaviour is related to f_H. The present thesis investigates this hypothesis using commercially available f_H biologgers (DST milli HRT, 13mm x 39.5mm, Star-Oddi, Iceland; http://www.star-oddi.com/) in two model species. Chapter one explores the relationship between f_H and parental care behaviour in nesting male smallmouth bass (*Micropterus dolomieu,* Lacépede 1802), and chapter two describes the relationship between f_H and dominance behaviour in sockeye salmon (*Oncorhynchus nerka,* Walbaum 1792) during spawning. Specifically, it was predicted that individuals that routinely use a higher proportion of their scope for f_H (i.e. f_H capacity) would utilize energy faster and therefore struggle to satisfy the metabolic demands of high energy behaviours associated with reproduction, compared to individuals that routinely use a low proportion of their scope for f_H.

Both selected model species are oviparous and reproduce in freshwater. Smallmouth bass were collected from Big Rideau Lake, Ontario, Canada (44.7706° N, 76.2152° W; Figure 1.1). This lake is part of the Rideau system, which is a series lakes and rivers joined by locks and channels, connecting the St-Laurence River in Kingston to the Ottawa River (Christie and Smol 1996). The system was created in 1832 to provide inland access to the Atlantic Ocean, but is currently primarily used for recreation. The Rideau system hosts a range of biodiversity including endangered species (e.g. eastern Musk Turtle, *Sternotherus odoratus*) and invasive species (e.g. zebra mussels, *Dreissena polymorpha*) (Government of Canada 2018). To help maintain native species richness, fishing sanctuaries have been implemented throughout the system to provide annual protection from anthropogenic fishing pressure (Ministry of Natural Resources and Forestry 2018).

Given the species richness, the Rideau system has also provided vast opportunities to research ecosystem, community and organism dynamics (e.g. Neff et al. 2003; Gravel and Cooke

2009; Locke et al. 2014). Smallmouth bass are an apex predator in the system (Aday et al. 2009), that feed on fish, insects, and invertebrates (e.g. crayfish) (Probst et al. 1984). However, smallmouth bass are also among the most popular sportfish, and conservation management programs have been implemented to protect the species from overexploitation (Cooke and Philipp 2009). Ontario regulations include the prohibition of fishing smallmouth bass from the Rideau system during the reproduction period (fishing season closed from December 15 to the third Saturday in June; Ministry of Natural Resources and Forestry 2018), at which point sexually mature smallmouth bass migrate to the littoral zone to spawn. The timing of smallmouth bass reproduction largely depends on the water temperature (ranging between 12.5 to 23.5°C; Graham and Orth 1986), and likely coincides with abundant food availability once the eggs mature to free-swimming fry (Cushing 1990). During reproduction, the males create a nest and the females select a male to spawn with (Ridgway 1998). A female can spawn with more than one male, and a male can spawn and guard the offspring from more than one female (Winemiller and Taylor 1982). Once eggs are deposited and fertilized, the subsequent parental care involves aerating the eggs and removing debris to prevent suffocation, and deterring predators and conspecifics (Cooke et al. 2006). Without parental care, the offspring will be predated on within minutes, depending on the lake system (Gravel and Cooke 2009). Smallmouth bass parental care is mono-parental (males only) and lasts between 2 to 5 weeks, at which point the offspring are free-swimming and have developed their own predator avoidance strategies (offspring mature faster in warmer temperatures; Cooke et al. 2006).

Unlike the smallmouth bass which are guarders, sockeye salmon are non-guarders (see Table 1.1 for definitions; Blumer 1979). They spawn in shallow fast flowing water, in temperatures less than 19°C, depending on the population (Hodgson and Quinn 2002). Sockeye salmon are a keystone, mid-trophic level species that serve as a food source for other fish (Ricker 1941), birds, and mammals (e.g. seals and bears) (Ricker 1941; Quinn 1999; Gende 2002; Hauser et al. 2008), and feed primarily on smaller fish and aquatic insects (Graynoth et al. 1986). They are an anadromous, semelparous species, meaning they start their reproduction period by migrating from the ocean to freshwater, and die after spawning (Groot and Margolis 1991). The distance and timing of migration varies between populations (e.g. Weaver, Chilko, Stellako, Gates populations), where each population has evolved different physiological thresholds correlated to the distance traveled (Eliason et al. 2011; Eliason and Farrell 2016). Once in the river, the spawning period lasts up to 5 weeks, but individuals live for approximately 10 days (these values were taken from Gates sockeye salmon data, and vary depending on the population; Lingard et al. 2013; discussed in Healey et al. 2003). During spawning, females create a nest (called a redd) by digging with their caudal fin and males compete for a reproductive female. Spawning occurs in high densities, and competition for a reproduction opportunity includes aggression behaviours, such as chasing (both sexes), charging (both sexes), posture displaying (dorsal fin erect and nose protruding the water surface – done by males), and lateral display (tense body, dorsal fin erect, spread pectoral and pelvic fins – done males only) to deter conspecifics. Dominant individuals are aggressive individuals that deter conspecifics from a territory or redd and undergo courting and spawning behaviours. Typically, dominant males have well developed secondary sexual characteristics and have a large body size allowing them to outcompete smaller males during physical competition and occasionally overrule female mate choice (Eseteve 2005). In contrast, subordinate individuals challenge a dominant individual with aggression behaviours in attempt to take over a territory or a redd. Subordinate males may also take on other alternative reproductive strategies including mimicking the appearance of a female to access a redd (sneaker male) or hover downstream from the dominant pair to intercept a reproduction opportunity once the eggs are released (satellite male). Courting behaviours include females probing the sediment in the redd with their anal fin to signal the oncoming of oviposition, and quivering done by both sexes to stimulate spawning. Once the eggs are deposited and fertilized, the female buries the eggs with gentle digs on the upstream rim of the redd. Occasionally the female will guard the redd to prevent the eggs from being uncovered by another fish, however mature fish die before the eggs mature to alevin.

Sockeye salmon data collection for this thesis took place on the Gates Creek spawning channel in D'Arcy, British Columbia (50.5481°N, 122.4832°W; Figure 1.2). The Gates Creek spawning channel is an approximately 2 km long, 8 m wide, and 0.5 m deep, man-made channel that meanders on the N'aquatqua First Nations reserve (Lingard et al. 2016). The sediment comprises of gravel and cobble, and the hydrology includes shallows fast-flowing riffles and deep pools. The channel was constructed in 1968 by the by the International Pacific Salmon Fisheries Commission (IPSFC) to enhance the Gates sockeye salmon population after it had been affected by major development projects in the early 1900's. Currently, the channel is being operated by the First Nations group N'Quatqua Fisheries Inc. (Department of Fisheries and Oceans Canada 2015), and information on the number of returning sockeye salmon and spawning status is relayed to Department of Fisheries and Oceans (Lingard et al. 2016). The Gates Creek spawning channel functions each year by intercepting spawning Gates sockeye salmon at a weir, where approximately 30-50% of the fish are diverted into the spawning channel, while the other 50-70% continue to migrate up the natural spawning creek (Lingard et al. 2013; 2016). Other fish species, such as the bull trout (*Salvelinus confluentus*) are excluded from the channel, to protect the sockeye salmon eggs from being predated (Lowery and

Beauchamp 2015; Lingard et al. 2013; 2016). Throughout the spawning period, sockeye salmon post-spawned corpses are counted, sexed, and the spawning status of the females is assessed according to the amount of eggs remaining in the body cavity. This system provides information on the population status permitting management of the Gates sockeye salmon population.

Table 1.1 Different reproductive strategies observed in teleost fish.

Parental care strategy	Description	Species example
Guarders	Involves the creation of a nest to contain the eggs and/or fry, cleaning and maintaining the nest to prevent eggs suffocation due to detritus, fungus or desiccation, feeding the offspring, and deterring conspecifics and nest predators (Balon 1975; Blumer 1979).	smallmouth bass; lingcod; three-spine stickleback; cichlids; lumpfish
Non-guarders	Includes broadcast spawners, open substrate spawners, and brood-hiders that deposit and actively bury fertilised eggs where they remain hidden without parent protection until hatched (Balon 1975; Blumer 1979).	capelin; northern pike; Atlantic salmon; sockeye salmon
Bearers	Involves carrying the embryos/offspring, internally or externally to protect them from predation and to supply their offspring with nutrients Examples include oral brooding, internal gestation, or carrying the brood in a specialised pouch (Blumer 1979).	seahorse; nursery fish

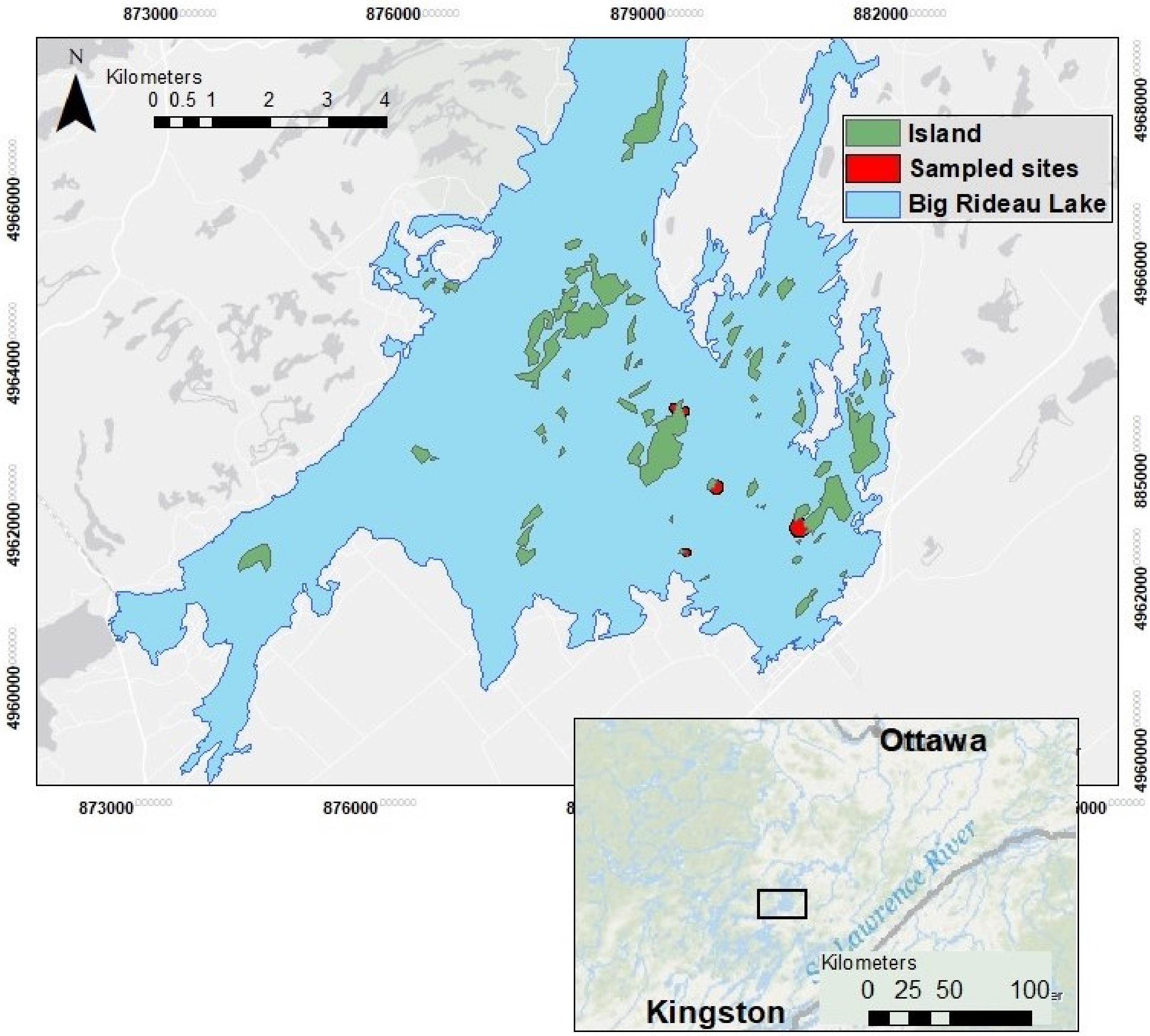

Figure 1.1 Map of Big Rideau Lake. The lake is part of the Rideau system, which is a series lakes and rivers joined by locks and channels, connecting the St-Laurence River in Kingston to the Ottawa River

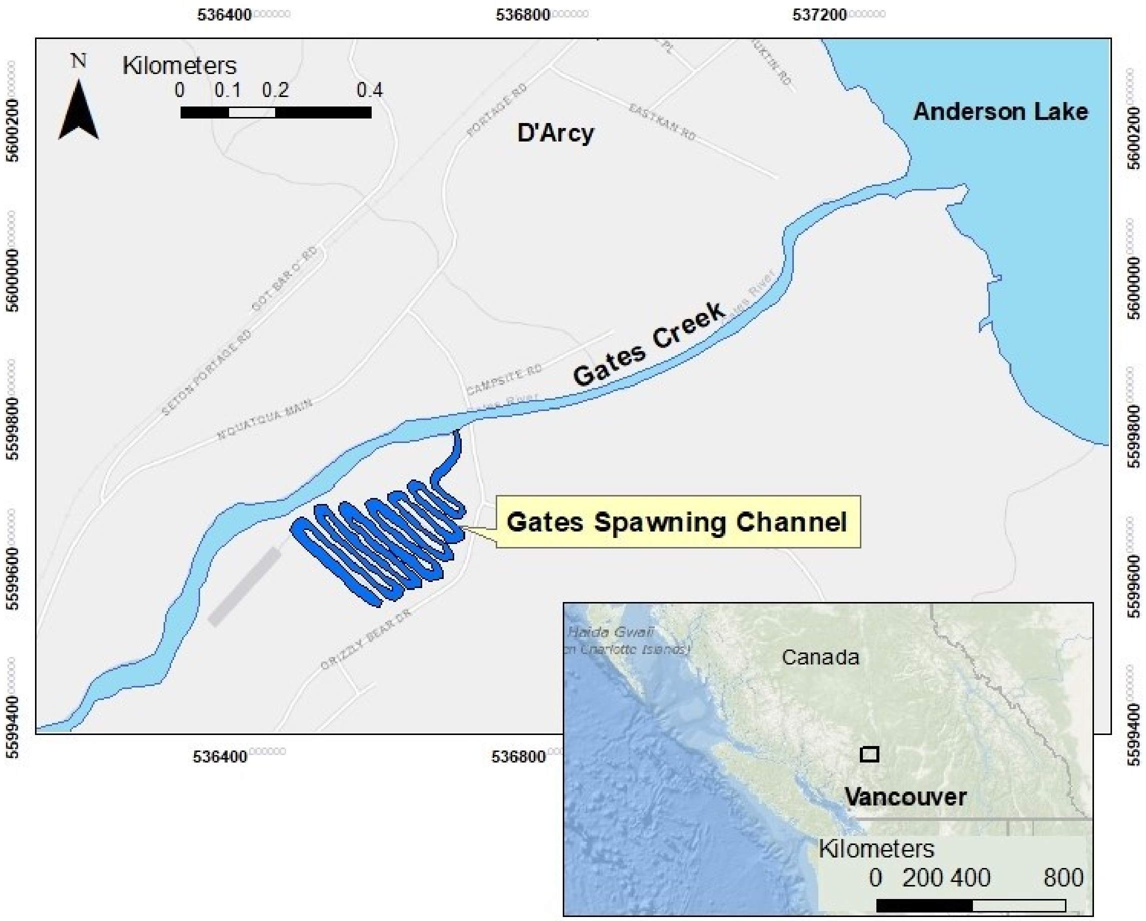

Figure 1.2 Map of Gate Spawning Channel, an approximately 2 km long, 8 m wide, and 0.5 m deep, man-made channel that meanders on the N'aquatqua First Nations reserve.

CHAPTER 2: Exploring relationships between cardiovascular activity and parental care investment in nesting smallmouth bass: a field study using heart rate biologgers

Abstract

Recent research in a variety of vertebrate taxa has found that cardiac function is a major limiting factor in the ability of animals to cope with physiological challenges, and thus is suggested to play an important role in mediating fitness and fitness-related behaviours in the wild. Yet, there is still a paucity of empirical assessments of the relationships between physiological performance and biological fitness in wild animals, perhaps due to challenges in measuring these metrics remotely. Using male smallmouth bass (*Micropterus dolomieu*) as a model, we tested for relationships between cardiac performance (measured using heart rate biologgers) and relevant fitness-related behaviours (assessed using videography and snorkeler observations) in the wild during the parental care period. Our results showed that smallmouth bass heart rates were not significantly related to any measured parental care behaviours (e.g., nest tending behaviours) except for individual aggression level. After accounting for the effect of water temperature variation on heart rate, we found no evidence for within-individual repeatability in heart rate among diel periods. There was, however, evidence of diel variation in heart rate; heart rate was higher during the day than at night. Although fitness is thought to be dependent on physiological capacity for exercise in wild animals, inter-individual variation in heart rate alone does not appear to relate to parental care behaviour in smallmouth bass. Further studies are required to confirm relationships between physiological performance and parental care investment to further reveal the apparently complex relationships between physiology, behaviour, and fitness in wild animals.

Introduction

Many wild vertebrates engage in parental care as a reproductive strategy to maximize offspring survival (Clutton-Brock 1991; Klug and Bonsall 2014). The parental care period is often highly physiologically demanding, frequently involving intense physical exercise, a lack of energy intake via foraging, and increased exposure/vulnerability to predators (e.g. Roff 1983; Bustnes and Erikstad 1991; Magnhagen 1991). If the energetic demand of parental care becomes excessive (exceeds a tolerance threshold), the parent may abandon the offspring, resulting in zero biological fitness for that reproduction period (Chellappa and Huntingford 1989). Researchers have rationalized strong relations between parental care investment (such as parental behaviour) and intraspecific physiological performance in wild animals (e.g. Ricklefs and Wikelski 2002; Westneat et al. 2011; Brownscombe et al. 2017), however the number of studies focusing on assessing these relationships is comparatively scarce. Instead, physiological diversity (i.e. variation in physiological performance between individuals) is often ignored (given inherent research challenges; Costa and Sinervo 2004), treated as noise, or controlled against in scientific literature (Bennett 1987; Spicer and Gaston 2009). Neglecting to supplement hypothesized relationships between physiological performance and parental care investment with validation studies has created gaps in the current understanding of the inter-relatedness between animal physiology, reproductive behaviour, fitness and, therefore, animal life history (Brownscombe et al. 2017).

Parental care is reasonably common among teleost fish (Blumer 1979). It is therefore not surprising that there have been numerous laboratory and field studies focused on understanding the factors that influence variation in individual fitness using teleost fish as models. Smallmouth bass (*Micropterus dolomieu*; Lacépède, 1802; centrarchidae) in particular have been the focus of much fieldwork on the physiology, energetics, behaviour, and individual reproductive success (reviewed in Cooke and Philipp 2009). During the four to six-week reproductive stage, male smallmouth bass create a nest, court with females, tend to the nest (e.g. fan eggs, remove debris), and protect their brood from predators and conspecifics (Ridgway 1998; Cooke et al. 2002a). Leading up to the reproduction period, energy is allocated towards gonad development and nest creation (Bevelhimer and Breck 2009). After reproduction, energy allocation shifts to supplement heightened activity associated with parental care behaviours (e.g. fanning; Hinch and Collins 1991; Cooke et al. 2002a), that requires enhanced oxygen delivery throughout the body (Cooke et al. 2004a; Cooke 2004). Parental care also requires constant vigilance, where if a bass leaves the nest to feed, the eggs would likely be predated within seconds or minutes, depending on the predation pressure of the system (Steinhart et al. 2004; Gravel and Cooke 2009). Therefore, during parental care, the male parent has limited feeding opportunities (Hinch and Collins 1991; Steinhart et al. 2004). Consequently, endogenous energy reserves decline during the parental care period (Mackereth et al. 1998; Gillooly and Baylis 1999) and parental males adjust their investment (e.g., locomotor activity dedicated to parental care; Ridgway 1998; Wiegmann and Baylis 1995; Cooke et al. 2002a) as part of a trade-off between current and future reproduction (Stearns 1989). It is therefore important for a parental male smallmouth bass to have a sufficient metabolic capacity to supply the body with the oxygen required to meet these energy demands during the prolonged parental care period, which often includes the need for short bouts of high intensity exercise (Priede 1977; Cooke 2004).

Research on various vertebrate taxa, including smallmouth bass, has suggested that energy demanding behaviours, such as parental care behaviours, rely strongly on aerobic scope and energy acquisition and utilization (Priede 1977; Reardon and Chapman 2010; discussed in Brownscombe et al. 2017). Cardiac function likely mediates parental care investment and fitness given its relationship with oxygen delivery and metabolism (Mirkovic and Rombough 1998) and circulation of signaling molecules (i.e. hormones), which are known to affect parental care (e.g. Dey et al. 2010). In fish, oxygen delivery to tissues, required to synthesize ATP (energy), is limited by the performance of the cardiovascular system (reviewed in Farrell et al. 2009; Eliason et al. 2013). Oxygen consumption increases with exercise and stress (Ji 1999; Farrell 2009; Eliason et al. 2013), which is regulated through adjustments in cardiac output and arteriovenous oxygen difference (Farrell et al. 2009; discussed in Eliason and Antilla 2017). Fish control cardiac output by modulating heart rate (f_H) and stroke volume. Yet in some species, including centrarchids, stroke volume has been found to remain relatively constant in various situations. For instance, when exhibiting temperature stress, smallmouth bass f_H and cardiac output increased while resting stroke volume remained unaffected (Schreer et al. 2001). These same trends were also reported in studies investigating the effects of seasonal changes (Cooke 2004) and exhaustive exercise (Schreer et al. 2001; Cooke et al. 2003a) on largemouth bass (*Micropterus salmoides*) cardiovascular performance, leading to the conclusion that largemouth and smallmouth bass are predominantly frequency modulators. Hence, f_H provides an effective real-time continuous proxy for metabolic performance in centrarchids (Schreer et al. 2001).

The apparent relationships between cardiovascular performance and metabolism, and physiological capacity for exercise and parental care behaviour has motivated the hypothesis that fitness and fitness-related behaviours are strongly influenced by cardiovascular performance (Franklin and Davie 1992; Cooke 2004; Farrell et al. 2008; Cooke et al. 2010). Previous research has shown that smallmouth bass (and other centrarchid species) undergo behavioural and physiological adaptations, including modulating f_H to enhance reproductive performance (Cooke et al. 2010). However, to our knowledge, no study has formally tested the hypothesis in free-swimming fish. Using free-swimming nesting smallmouth bass as a model species, this study tested the hypothesis that f_H is related to parental care behaviour investment. More specifically, we predicted that since the cost of parental care increases with decreasing scope for f_H (lower capacity for frequency modulation), individuals that routinely utilize a lower proportion of their scope for f_H (calculated as peak f_H – resting f_H) would have a lower relative energy expenditure rate. This would be indicative of a higher metabolic capacity to cope with the energetic requirements associated with parental care activity as the brood matures and endogenous energy stores decrease. In contrast, individuals that routinely utilize a higher proportion of their scope for f_H would either have poor individual physiological capacity or be subject to elevated pressure from parental care burdens (e.g. temperature stress and nest predators), and will be more likely to exhaust their energy, invest less time on nest tending parental care behaviours, abandon their nest, and hence be less reproductively fit than individuals operating at a lower scope for f_H.

Methods

Fish were collected under the Ontario Ministry of Natural Resources licence #1079390, and the study was designed in accordance with the Canadian Council of Animal Care protocol, under the permit number BT-026 administered through the Carleton University Animal Care Committee.

Day 1

Nest identification

From May 23 to 28, 2016, snorkelling surveys identified 24 nesting male smallmouth bass (mean L_T 422mm ± 1.6mm) on nests in Big Rideau Lake (Ottawa, Ontario, Canada, 44.7706° N, 76.2152° W). Nest size was estimated by scoring the nests on a scale from 1-5, where 1 was a nest with few eggs and 5 was a nest with thousands of eggs (Algera et al. 2017a).

Zuckerman (2014) reported that fish with a nest score less than 3 and egg stages greater than 4 days (out of approximately 6 days of egg stage and 22 days of parental care; Cooke et al. 2002a) were more likely to abandon. Therefore, to reduce the risk of smallmouth bass abandoning their nest with the f_H logger, only fish with a nest score of 3 or more, and an egg stage of 4 days or less were used in this study. Nests that met the criteria were labelled using weighted numbered tags.

Surgery and instrumentation

Smallmouth bass were collected off their nest by angling coupled with assistance from the snorkeler providing hand signals to insure the correct bass was caught. Fish were landed within 20 s after hookset to reduce anaerobic exercise and stress associated with angling (Cooke et al. 2003a; Lawrence et al. 2018), and brought into the boat using a rubberized net to avoid injury. Once un-hooked, bass were placed onto a surgery table with water being continuously pumped over the gills, and were electro-sedated using fish handling gloves (Smith-Root, Inc., Washington, USA, http://www.smith-root.com; 10mA). An approximately 5 cm longitudinal incision was made half-way between the pectoral and pelvic fins, posterior to the pericardial membrane. f_H loggers (DST milli HRT, 8g, 13mm x 39.5mm, Star-Oddi, Iceland; http://www.star-oddi.com/) were inserted immediately posterior of the pericardial membrane and sutured to the ventral musculature (PDS II polydioxanone suture; violet monofilament, 3-0). f_H loggers were programmed to record f_H at 100 Hz every 2 minutes, and ECG every 1.5 hours to validate f_H readings. The incision was closed using four to five square knot sutures, and the total length of the fish was measured, after which the fish was recovered and then released close to the nest. Throughout the entire process, a snorkeler was protecting the nest from predators and conspecifics until the adult bass returned to the nest and resumed nesting behaviours. The loggers began collecting data at midnight on the same day of insertion.

Day 2-3

Behaviour footage and predator exposure

One hour of behavioural footage was collected using a GoPro (HERO3+) supported by a 30 cm tall stand, placed 1 m from the edge of each nest. The following day, a GoPro was returned to each nest for another 50 minutes of behavioural footage, after which a pumpkinseed (*Lepomis gibbosus*; Linnaeus, 1758; L_T = 130-150 mm) in a 2 L clear, glass jar with open water circulation was placed on the edge of the nest, opposite side from the GoPro, for the subsequent 10 minutes. The latter provided behavioural footage in the presence of a nest predator, used to assess the parental male aggression score (Hanson et al. 2009). At the end of the hour, both the GoPro and the predator were removed.

Day 4

f_H logger removal

Again, with the assistance of a snorkeler, the instrumented smallmouth bass were re-angled off their nest. If the bass could not be recaptured from the boat, the snorkeler angled the bass given their better perspective of the fish. As soon as the bass was caught, the snorkeler placed a cage over the nest to prevent the eggs from being preyed on. Using a lithium heparinized vacutainer and a 21 gauge needle (B.D. Vacutainer, NJ, USA), approximately 2 mL of blood was sampled, within 3 minutes of capture (Lawrence et al. 2018), from the caudal vasculature for hematocrit analysis. This was immediately followed by a 3 minute forced exercise period, which involved placing the bass in a 115 L (90 cm diameter × 30 cm height) basin filled with fresh lake water, where a researcher continuously attempted to grab the caudal peduncle to cause the fish to burst swim. The bass was then placed into a cooler (66 cm × 34 cm

× 31 cm) for one hour. Water changes were made continuously over this time frame to avoid exposing the fish to hypoxia during recovery. The purpose of the chase and one hour wait was to obtain an estimate of the fish's maximum f_H (Cooke et al. 2002b).

One hour after the fish was placed into the cooler, the f_H logger was removed using the same surgical set-up and electro-anesthesia as implantation. The incision was re-opened to retrieve the logger, and sealed in the same manner. The fish were released close to the nest, at which point the cage covering the eggs was removed and the snorkeler protected the nest until the bass returned and resumed nesting behaviours.

Hematocrit was separated using heparinized capillary tubes (75 mm Drummond Hemato-Clad, ammonium heparin, Toronto, ON, Canada) centrifuged for 5 minutes at 8000 g (Clay Adams^{TM}, NJ, USA). Blood content proportions were measured to the nearest mm via a ruler.

Day 5 - onward

Monitoring egg development

Throughout the study and until June 6th, 2016, a snorkeler monitored the nest every other day for brood success. A nest was deemed successful if the offspring matured to free-swimming fry.

Behaviour data collection

Using JWatcher (Blumstein et al. 2000; http://www.jwatcher.ucla.edu/), video footage from day 2 and day 3 were analysed separately. For both days of nesting behaviour footage, only footage from minute 20 to minute 40 in the hour-long behavioural footage was analyzed. This allowed the nesting bass to acclimatize to the GoPro, and ensured that the boat and the snorkeler were not present when behaviour was being recorded. The time and duration of nesting behaviours were recorded and organized into one of three behavioural categories: nest tending –

actively maintaining the nest (removing debris, fanning eggs); nest guarding – staying on or near the nest but not conducting any nest tending behaviours (hovering over the nest or within 1 m from the nest, swimming around the nest); and nest defending – deterring predators and conspecifics (hovering with dorsal fin erect, actively chasing predators). Additionally, the number of nest predators and conspecifics that appeared in the footage were recorded.

Similarly, individual aggression score was assessed by adding the time that the bass' mouth was on the jar (rounded to the nearest second), the number of strikes on the jar (mouth bumps the jar), and the number of yawns (Hanson et al. 2009; Zolderdo et al. 2016). Additionally, the time until the first attack, measured from when the jar was dropped to when the first aggression behaviour occurred, as well as the time spent swimming around the jar, and the time spent chasing other predators and conspecifics were also recorded.

Data Processing

A total of 22 nesting smallmouth bass were tagged, where 3 fish abandoned with the logger and 2 fish had failed f_H records. Therefore, 19 fish were used for behavioural analysis and only 17 fish were included in the f_H analyses. Temperature (measured by the f_H loggers) changed by ~7.5°C during the eight-day study period. Using RStudio (v. 3.2.3, RStudio Inc., Boston, MA, USA; https://www.rstudio.com/) a simple linear regression model was employed to investigate relationships between temperature (rounded to the nearest degree Celsius) and f_H, where the slope of the relationship provided an estimate for Q_{10}. Since temperature is related to f_H (Reynolds 1977) and behaviour (Cooke et al. 2003c), the effect of temperature on f_H and fish behaviour was detrended by determining the residuals from the linear regression between f_H and temperature, and the linear regression between behaviour and temperature (Jakob et al. 1996), and using these residuals in further analyses.

f_H data was analysed by determining the routine, resting, max, min, and scope for f_H (max f_H – resting f_H). Occasionally, the 3 min chase did not yield a peak in f_H, likely due to poor swimming performance during the chase. Therefore, max f_H was determined as the highest f_H record throughout the f_H trace. Routine f_H was calculated as the average of the f_H trace excluding when the fish was captured and chased. Resting f_H was calculated as the lowest 10th percentile f_H values, also excluding when the fish was captured and chased. Finally, the percentage of routine f_H within scope for f_H (R%f_H) was calculated as ((routine-resting) ÷ scope for f_H) × 100. This allowed comparison between fish according to the proportion of the scope for f_H that was routinely being used. For all individuals, f_H measurements included two days and two nights.

Statistical Analyses

A one-sample t-test confirmed there was no difference in time spent on each behaviour between day 1 and day 2 (difference between times on each behaviour did not significantly differ from 0; p-value = 0.99 in all cases). This was confirmed with a Spearman's rank order correlation analysis where the time spent on each behaviour had a positive correlation between day 1 and day 2 (guarding: *rho* = 0.70, p-value <0.001; tending: *rho* = 0.70, p-value = 0.0017, and defending: *rho* = 0.41, p-value = 0.054). The positive relationship between behaviour on day 1 and behaviour on day 2 supports the results that parental care behaviour was repeatable in the present study. Therefore, further analyses used the average time spent on each behaviour, predator count during the behavioural footage, and aggression score between day 1 and day 2. A linear mixed effect model was fit to compare the time spent on each parental care behaviour (time as the response variable and behaviour interacting with egg score as the predictor variables), where individual was treated as a random effect to account for dependencies. The model was simplified using the drop1 command in R, and significant predictors were assessed using p-values based on the F-distribution. For significant variables, a Bonferroni adjusted Tukey post-hoc analysis was used for pairwise comparisons. Given the response variable, R%f_H is a proportion and continuous, a beta regression (Kieschnick and McCullough 2003; *betareg* package, Zeileis et al. 2016) model with R%f_H as the response variable and nesting behaviours, aggression, fish length, egg score, and hematocrit as covariates, was used to relate f_H to parental care investment. To avoid overfitting the model, random forest analysis (*random forest* package, Liaw and Wiener 2015) was used to identify the five covariates with the greatest influence on f_H variability that were to be included into the model according to %IncMSE, and stepwise model selection was conducted using AICc to account for the small sample size (Table 2.1).

To determine whether f_H frequency modulation related to parental care behaviour, a linear mixed effect model was used to relate f_H (recorded every 2 minutes) to nesting behaviours, aggression, fish length, egg score, and hematocrit. Again, random forest analysis was used to select the top five covariates to avoid overfitting the model. Three linear mixed models were fit, all with individual fish as the random effect, where model 1 was set to have random intercepts, model 2 was set to have random slopes and intercepts, and model 3 was set to have random slopes. All models were fit using the *nlme* package in R (Pinheiro et al. 2014). A one-way ANOVA was used to select the best model (model 3, random slopes) according to the lowest BIC value (model 1 = 162146.2; model 2 = 161958.6; model 3 = 161958.4). Stepwise model reduction using the drop1 command (Chambers 1992) suggested the model could be further reduced to only include time as a covariate (Table 2.2).

Finally, individual rank order repeatability in R%f_H between individuals was compared using Spearman's rank correlation to determine if f_H was repeatable overtime (2 days and 2 nights) during the nesting period. Diel patterns in f_H were assessed using a linear mixed effect model with individual as a repeated measure. Since only 2 fish abandoned their nest and did not have offspring attain free-swimming fry, we could not statistically relate f_H to successful brood development. Instead, the relationship between R%f_H and parental care performance and brood success was visually compared for potential trends. All statistical analyses were conducted using RStudio.

Results

Characterizing f_H

An example of a smallmouth bass f_H trace during 64 hours of parental care is presented in Figure 2.1. In all cases, hematocrit levels were above the poor condition threshold of 20% (mean = 31.0% ± 2.2%; Fränge et al. 1992; Gallaugher and Farrell 1998). Nesting smallmouth bass had an average routine f_H of 47.4 beats min^{-1} ± 1.6 beats min^{-1}, with an average resting f_H of 24.8 beats min^{-1} ± 1.7 beats min^{-1}, and a minimum f_H and maximum f_H of 17.9 beats min^{-1} ± 0.9 beats min^{-1} and 124.6 beats min^{-1} ± 2.7 beats min^{-1}, respectively (n =17; water temperature ranged from 16°C-23°C).

There was a significant positive correlation between temperature and f_H ($p < 0.001$, $r^2 = 0.97$), where f_H increased on average by 4.41 beats min^{-1} (8.22% ± 2.0%) with every degree Celsius increase between 16°C to 23°C (Figures 2.1 and 2.2). When corrected against temperature effects, the average scope for f_H changed from 99.9 beats min^{-1} ± 2.2 beats min^{-1} to 101.1 beats min^{-1} ± 2.8 beats min^{-1}. Hence, on average, the routine f_H of nesting male smallmouth bass operated at 18.13% ± 1.06% of the scope for f_H without temperature correction, and at 23.6% ± 1.2% of the scope for f_H after temperature correction, with a 20% range for both cases (Figure 2.3).

Relationship between f_H and nesting behaviour

During the nesting period, male smallmouth bass spent the greatest proportion of their time guarding the nest (56.7 % ± 3.0) followed by tending to the nest (34.6% ± 3.2%), which was six fold and four fold greater than the time spent defending the nest from predators and conspecifics (8.7% ± 1.2%), respectively ($p < 0.001$ for all cases; Figure 2.4). Regardless of the observable variation in interindividual f_H and parental care behaviour (Figures 2.3- 2.5), random forest analysis yielded no predictability power between f_H (specifically R%f_H) and parental care behaviours, fish condition, and nest predation pressure (percent variance explained = -41.98%). A beta regression model containing the five most influential covariates (according to the random forest analysis: nest defending, egg score, time to first attack, time spent swimming around the jar, and aggression score; AICc: -26.09) yielded no significant relationship between parental care behaviours and R%f_H (all p-values > 0.05; Table 2.1). A model including only aggression score as a covariate (according o the lowest AICc: -49.08) yielded a significant relationship between R%f_H and aggression score (p-value = 0.0075; Figure 2.6). Since only two f_H loggers could be retrieved from fish that abandoned, the relationship between f_H and the likelihood of nest abandonment could not be statistically compared. However, there was an apparent relationship between f_H and brood success (Figure 2.5C), where the two fish that abandoned their brood prematurely were among the fish with a higher routine f_H, resting f_H half), minimum f_H (all within the top fiftieth percentile), and the lower maximum f_H (lowest quarter percentile). Furthermore, smaller nest scores appeared to coincide with fish that had a higher resting f_H and a lower R%f_H (Figure 2.5E). However, for both apparent trends, no significant relationship was detected (all p-value > 0.05). Similarly, there was no detectible relationship between f_H frequency modulation patterns and nesting behaviours (p-values > 0.05; Table 2.2). The only significant relationship (p-value = 0.028) suggested that f_H frequency modulation may decrease as the nesting period progresses (Figure 2.7).

Although behaviour remained relatively consistent through time (see Methods section), f_H was not repeatable between days or nights (p-value > 0.05). However, nesting smallmouth bass f_H showed diel variation. More specifically, R%f_H was 10% higher during the day than at night (p-value = 0.003; Figure 2.8).

Discussion

The present study tested the hypothesis that cardiac performance mediates fitness and fitness related behaviours (Franklin and Davie 1992; Cooke 2004; Farrell et al. 2008; Cooke et al. 2010). It is also the first study to monitor cardiac function in free-swimming fish during parental care. Despite the intraspecific variation in f_H recorded in this study, and the apparent relationship between metabolic performance and fitness presented in recent literature (Ricklefs and Wikelski 2002; Westneat et al. 2011; reviewed in Brownscombe et al. 2017), there was no relationship between individual percent usage of scope for f_H and reproductive investment. A marginally non-significant positive relationship between R%f_H and parental male aggression level was detected (Figure 2.6), however this did not affect parental care behaviours, specifically nest defending, nest tending and nest guarding investment. Hence, our results partially support the hypothesis: although f_H is related to an individual's aggression level, the lack of relationship between f_H and parental care behaviours suggests that f_H alone does not appear to be directly related to parental care investment in smallmouth bass.

The recorded f_H conforms with the rates reported in previous literature (Schreer et al. 2001 (16°C = 34.5 beats min^{-1} ±2.7 beats min^{-1} and 20°C = 52.8 beats min^{-1} ± 2.5 beats min^{-1} for routine f_H and scope for f_H ranging between 1.8 and 2.6 fold); Cooke 2004 (21°C = ~49 beats

min^{-1} for routine); Cooke et al. 2010 (21°C= ~50 beats $\text{min}^{-1}$for routine); Cooke et al. 2004b (in largemouth bass (*Micropterus salmoides*) 17°C = ~48 beats min^{-1}, 21°C = ~50 beats min^{-1}, and 25°C = ~ 60 beats min^{-1} for routine f_H; 17°C = ~80 beats min^{-1}, 21°C = ~90 beats min^{-1}, and 25°C = ~ 120 beats min^{-1} for max f_H), measured within the same temperature range (16-23°C). Differences in f_H records compared to literature values are likely due to differences between the f_H loggers used in the present study compared to other devices, where ECG was not recorded to validate all f_H readings. Therefore, analysis was conducted using all recorded f_H data within range (less than 130 beats min^{-1}) and repeated using cleaned traces (maximum f_H = 95.4 beats min^{-1} ± 2.1 beats min^{-1}; data not provided). Both methods yielded the same results.

Although f_H was only monitored for up to 2.5 days during the egg developmental stage, f_H fluctuated throughout the period (Figure 2.3). Previous research has shown that environmental factors, activity, and hormones can affect fish f_H (discussed in Hoar et al. 1992 and Farrell 1993; Vornanen 2017). For instance, cardiac output increases with temperature, where, in teleost fish, the pacemaker rate increases with acute temperature changes such that the Q_{10} for f_H is ≥ 2.0 (Farrell 1992). In the case of nesting smallmouth bass, we showed that f_H increased by ~8% with every 1°C increase (Figure 2.2). Temperature has also been shown to strongly influence the timing of smallmouth bass reproduction (Graham and Orth 1986), however during parental care, lack of patterns between temperature variation and nesting behaviour reported in a previous study suggests smallmouth bass adjust their energy expenditures according to their environment (Cooke et al. 2003a). This adjustment in behaviour is likely partially responsible for the observed increase in f_H with temperature as well.

Irrespective of temperature, physiological variation persisted between individuals (Figure 2.5), including interindividual R%f_H (Figure 2.3). Although it has been demonstrated that morphological variation, specifically male size (Wiegmann and Baylis 1995; Algera et al. 2017a), is positively related to brood size and fitness, there was no relationship between brood size, male size, and f_{H} in the current study. Nest abandonment rates appeared to be higher in nesting males with higher resting f_{H}, routine f_{H}, minimum f_{H} and maximum f_{H} (Figure 2.5), however these apparent trends could not be statistically tested and may be artifacts of varying physiological states between individuals or small sample size; further investigations are required to confirm these trends. Additionally, previous literature reported a 60% higher metabolic rate (Hinch and Collins 1991) and enhanced cardiovascular performance (Cooke et al. 2010) in nesting smallmouth bass compared to non-nesting individuals, while Gillooly and Baylis (1999) demonstrated that energy expenditure during parental care is positively related to reproductive success. These trends are likely due to the enhanced energetic demands of parental care behaviours and heightened activity (Cooke et al. 2002a; Cooke et al. 2004a; Bevelhimer and Breck 2009). The positive relationship between R%f_{H} and the aggression score in the present study supports the findings in previous literature (e.g. Ballew et. al. 2017 – suggesting individual boldness is related to fitness), and further supports the hypothesis that smallmouth bass are frequency modulators, where individuals with a greater capacity to frequency modulate can cope with a greater predator burden and invest more and energy deterring predators and conspecifics (Figure 2.6). This also suggests that smallmouth bass nesting in lakes with higher predation pressure may have a stronger relationship between f_{H} and parental care behaviour. Irrespective of this trend, the smallmouth bass in the present study mostly exhibited nest tending and nest guarding behaviours, with the least amount of time spent on nest defending behaviours. This was likely because of the low predation pressure in Big Rideau Lake (Gravel and Cooke 2009), allowing the fish to allocate more time on their nest and less time deterring predators and conspecifics. Since nest tending behaviours, such as fanning and removing debris are highly energetically demanding (Lachance and Fitzgerald 1992; Cooke et al. 2002a), it is not unexpected that the time was split with nest guarding behaviours, which are less energetically demanding. However, given the lack of relationship between f_H and parental care behaviour, the variation in f_H cannot be attributed to interindividual variation in parental care activity. Instead variation in nesting behaviour could simply be a product of behavioural syndromes (Sih et al. 2004; Bergmüller 2010; Conrad et al. 2011), other endocrine factors such as stress hormones (Dey et al. 2010; Zolderdo et al. 2016; Algera et al. 2017a; Brownscombe et al. 2017), or because this study only involved fish that managed to attract a female and reproduce, while excluding fish that failed to spawn. Further studies are required to investigate these relationships using individuals that succeeded in spawning and individuals that abandoned. Lastly, given that cardiac output is a product of f_H and stroke volume (Farrell 1993; Vornanen 2017), we cannot rule out the possibility that aerobic scope and exercise influence nesting smallmouth bass behaviour without verifying this relationship using all cardiovascular parameters in future studies.

Although the present study could not confirm a relationship between individual f_H and parental care performance in nesting smallmouth bass, this was the first study to monitor f_H in nest guarding fish in the wild. Individual f_H profiles suggests f_H may decrease as the parental period progresses and follow an consistent diurnal pattern overtime, with no consistency among individuals. Due to the trade-off between the longer f_H monitoring period and the increasing risk of smallmouth bass abandoning the nest with the f_H loggers and therefore the f_H data, f_H was only monitored for up to 2.5 days. Hence, it is likely that the observed inconsistency and decreasing trend in f_H could be a result of the fish still recovering from the surgery, small sample size, or short monitoring duration. Nevertheless, it is known that f_H is modulated by environmental factors and by the supply or demands of oxygen and metabolites (Stecyk et al. 2011; Eliason and Antilla 2017; Vornanen, 2017). Given that the present study showed fluctuations in nesting smallmouth bass f_H that cannot be explained by temperature, nesting behaviour, individual aggression level, size, nor condition (hematocrit), it is likely that variation in f_H is predominantly related to another factor or a combination of factors that were not included in this study, such as photoperiod, hormones, blood plasma constituents (Hoar et al. 1992; Farrell 1993; Vornanen 2017), predation risk on the parent male (Cooke et al. 2003b), noise pollution (Graham and Cooke 2008), or nest location (Lachance and Fitzgerald 1992). Further research should investigate whether f_H becomes repeatable and continues to decrease after 1-2 days of recovery and a longer monitoring period.

The observed diel pattern in the nesting smallmouth bass f_H profiles (Figure 2.8) confirms that f_H is less dependent on parental care behaviour given that previous research has demonstrated there is no diel variation in nesting smallmouth bass parental care behaviour (Hinch and Collins 1991; Cooke et al. 2002a). This trend could be due to diel pattern in activity (routine and burst swimming), where activity is higher during the day than at night, however these differences in activity are small (Algera et al. 2017b). Since the effect of temperature on f_H was corrected against, the magnitude of the diel variation in f_H (10%) suggests that other extrinsic and intrinsic factors may also affect smallmouth bass metabolic performance. Previous research has shown diel cycles occurring in blood plasma constituents, such as cortisol, which increases with the onset of light and continues to increase with activity (Pickering and Pottinger 1983; Meier 1984; Cousineau et al. 2014). Although cortisol drives other endocrine and metabolic rhythms, there is a wide variation in the response of f_H and other cardiovascular parameters (Pickering and Pottinger 1983). Furthermore, Algera (2017b) showed that experimentally elevated cortisol levels in nesting smallmouth bass decrease fitness by reducing locomotor activity, burst swimming, routine swimming, while increasing resting behaviour. Other hormones have also been reported to exhibit diel rhythms in teleost fish (e.g. prolactin, melatonin, insulin, gonadal steroids), as well as plasma electrolytes ([Na^+], [Ca^{2+}], [Mg^{2+}], [K^+]), plasma metabolites (e.g. glucose – observed in sea bass during a 7 day fasting period; McDonald and Milligan 1992), some of which act on f_H by modulating the relative levels of adrenergic and cholinergic tones causing f_H to deviate from the intrinsic pacemaker frequency (McDonald and Milligan 1992; Farrell 1993; Vornanen 2017). The combination of diel cycling hormones, plasma electrolytes, and plasma metabolites likely drive the observed diel pattern in nesting smallmouth bass f_H. Hence, this further supports the hypothesis that f_H is predominantly influenced by endogenous and environmental factors and less influenced by behaviour and activity.

The approach used in the present study allowed smallmouth bass f_H and parental care behaviour to be monitored in the natural environment. However, the effects of the surgery cannot be ignored. To account for post-surgery recovery, analysis using only day 2 f_H was conducted, confirming the reported relationships (data not shown). Additionally, it is worth noting that the diel pattern becomes less distinct as time progresses (Figure 2.8), suggesting the trend may be due to environmental variability or an artifact of the surgery. The development of less invasive methods for monitoring f_H in free-swimming fish are required to better investigate these trends.

Conclusion

In conclusion, smallmouth bass show vast individual physiological and behavioural variation during parental care, however f_H was not directly related to parental care behaviour in our study. In fact, f_H appears to be unrelated to behaviour during the nesting period, as shown by the diel variation in f_H while behaviour remained unchanged. Despite the hypothesis relating physiological performance and fitness, this study is among many others failing to support this relationship (e.g. Wiegmann and Baylis 1995; Hatfield and Schluter 1999; Hanson et al. 2009; discussed in Spicer and Gaston 2009, and Brownscombe et al. 2017). While evidence that activity is fundamental to parental care performance in smallmouth bass (Gillooly and Baylis 1999; Cooke et al. 2002a) indeed implies a relationship between metabolic performance and fitness, the mechanisms remain elusive. Further research on a variety of vertebrate taxa is required to understand the complex relationship between intraspecific variability in physiological performance and fitness, which can ultimately provide new platforms for which animal energetics, behaviour, life history, and population dynamics can be studied.

Table 2.1 Output for beta regression model relating percent routine f_H within scope for f_H (R% f_H) to the five most influential covariates according to the random forest analysis. Nest defending = proportion of time parental male spent deterring predators and conspecifics; Egg score = estimated nest size; Time to first attack = time until first aggression behaviour during the predator simulation measured from when the jar was dropped; Aggression score = number of times the bass made contact with the jar or yawns during the predator simulation; Time swimming around jar = time that the parental male bass spent facing the jar without attacking during the predator simulation. Statistical analysis was conducted on 17 individuals and evaluated at an $\alpha = 0.05$.

Covariate	Estimate	Std. Error	z value	P(>|z|)
Intercept	-1.0	0.30	-3.51	0.0004
Time to first attack	-1.7e-5	1.6e-5	-1.1	0.29
Aggression score	0.00052	0.0020	0.26	0.80

Table 2.2 Output for linear mixed effect model simplification using log ratio tests to compare the model against reduced models via the 'drop1' command in RStudio. Time swimming around jar = time that the parental male bass spent facing the jar without attacking during the predator simulation; Aggression score = number of times the bass made contact with the jar or yawned during the predator simulation; Time = duration that heart rate (f_H) was monitored; Nest guarding = proportion of time parental male spent on or near the nest but not conducting any nest tending behaviours; Nest tending =proportion of time parental male spent on nest tending behaviours. Colon represents an interaction between covariates. Statistical analysis was conducted on 17 individuals and evaluated at an α = 0.05. Significance is identified in bold.

Covariate dropped	df	Likelihood Ration Test	AIC	Pr(>Chi)
-	-	-	161809	-
Time	1	4.8	161812	**0.028**
Aggression score	1	1.5	161808	0.22
Time swimming around jar	1	1.9	161809	0.17
Nest tending:Nest guarding	1	0.56	161808	0.45

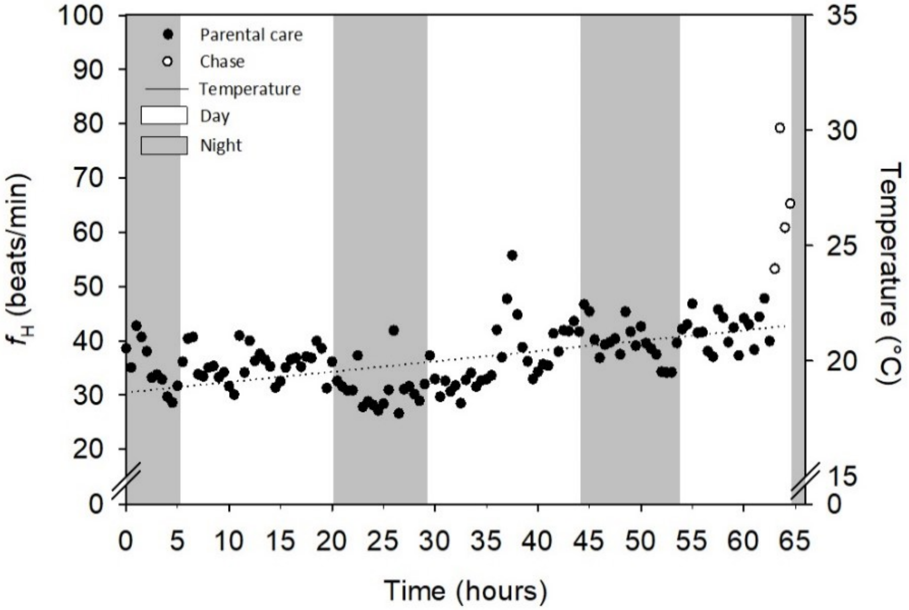

Figure 2.1 Scatterplot of an individual fish f_H (beats min^{-1}) averaged for every half hour over a 64 hour period. Black dots represent f_H during parental care. White dots represent f_H during the recapture and chase. Dashed line is the regression line for the water temperature. White bands represent daytime (5:30) and grey bands represent night (20:30).

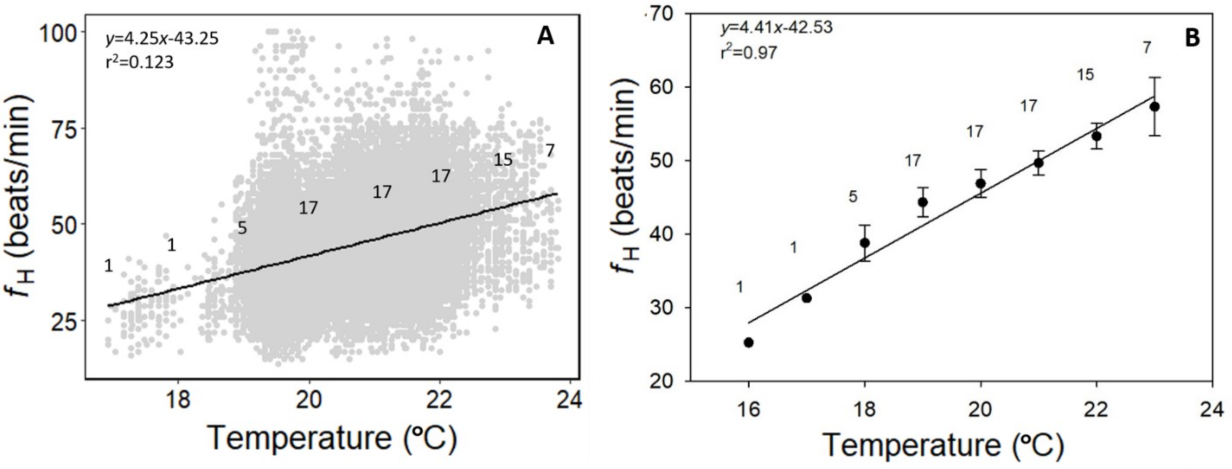

Figure 2.2 Regression plot showing A) raw and B) mean f_H of 17 nesting male smallmouth bass in response to water temperature increase in 1°C increments. The regression line shows that f_H increases according to the equation $f_H = 4.25$ (temperature °C)-43.25, with a coefficient of determination (r^2) of 0.123, p-value > 0.05, but on average f_H increases according to the equation $f_H = 4.41$(temperature °C)-42.53, with an r^2 of 0.97, p-value < 0.001. Error bars represent the standard error for each mean. Numbers over each point describe the number of individuals exposed to each temperature.

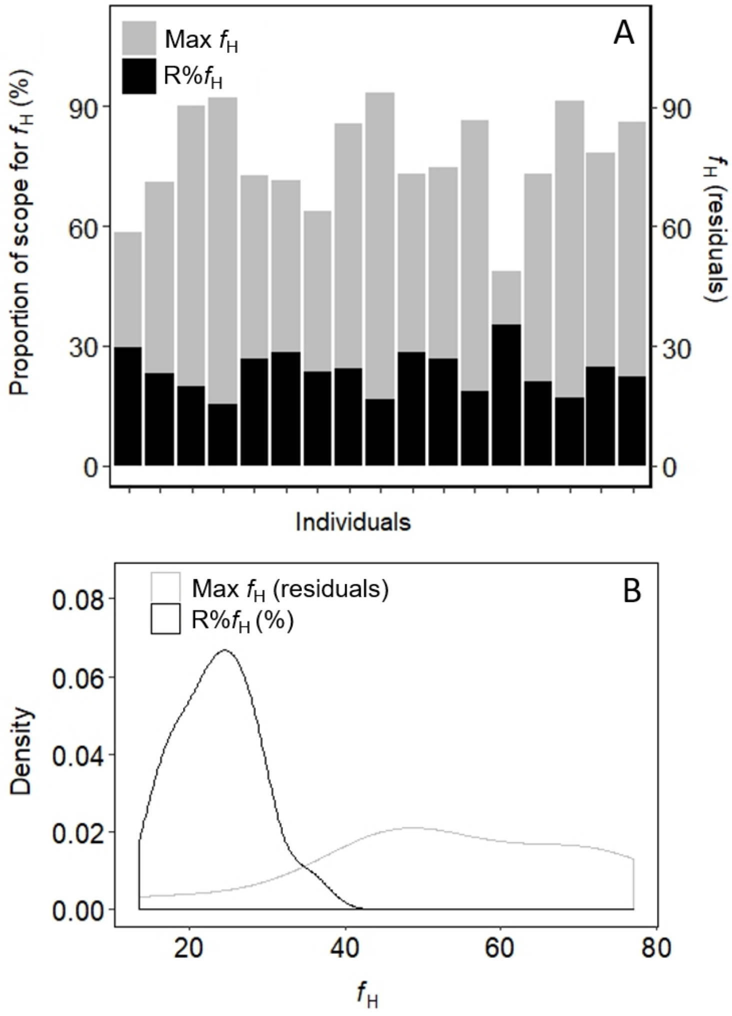

Figure 2.3 Double bar plot (A) and frequency distribution plot (B) showing intraspecific variability and range in f_H in nesting male smallmouth bass. Double bar plot (A) shows the recoded individual variability in f_H of nesting male smallmouth bass. The recorded routine f_H within scope for f_H (R%f_H) (%) of individual nesting smallmouth bass (the left axis) is presented in grey. The recorded maximum f_H (in residuals to correct for the influence of temperature of f_H) of each individual (right axis) is presented in black. Density distribution plot (B) shows the frequency of individual variation in percent R%f_H (%) (black) and the maximum f_H (residuals) (grey) in nesting smallmouth bass after temperature correction (n=17).

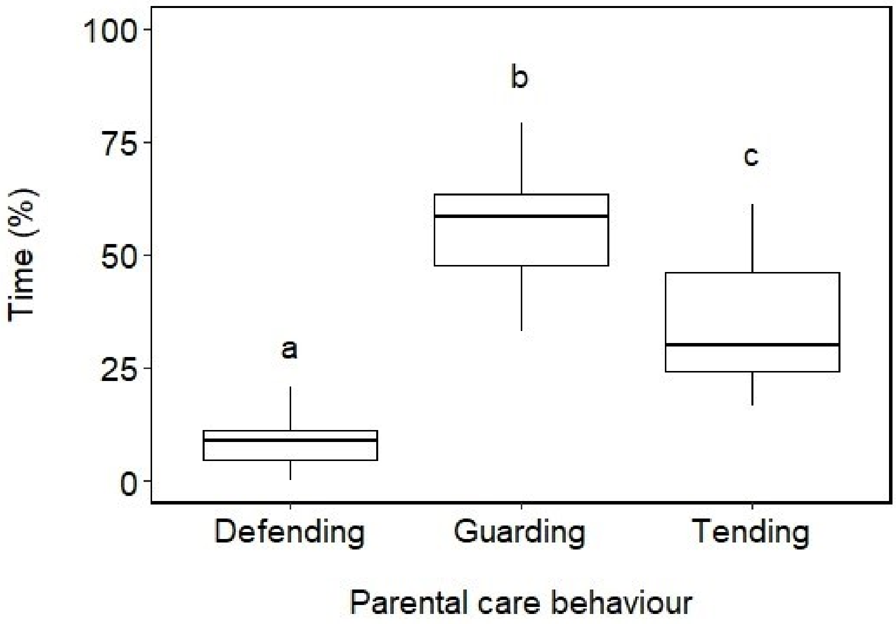

Figure 2.4 Percentage of time male smallmouth bass spent on each parental care behaviour over the forty minutes of monitored behaviour (n=19). Different letters show significant differences between groups ($p < 0.001$ for all cases).

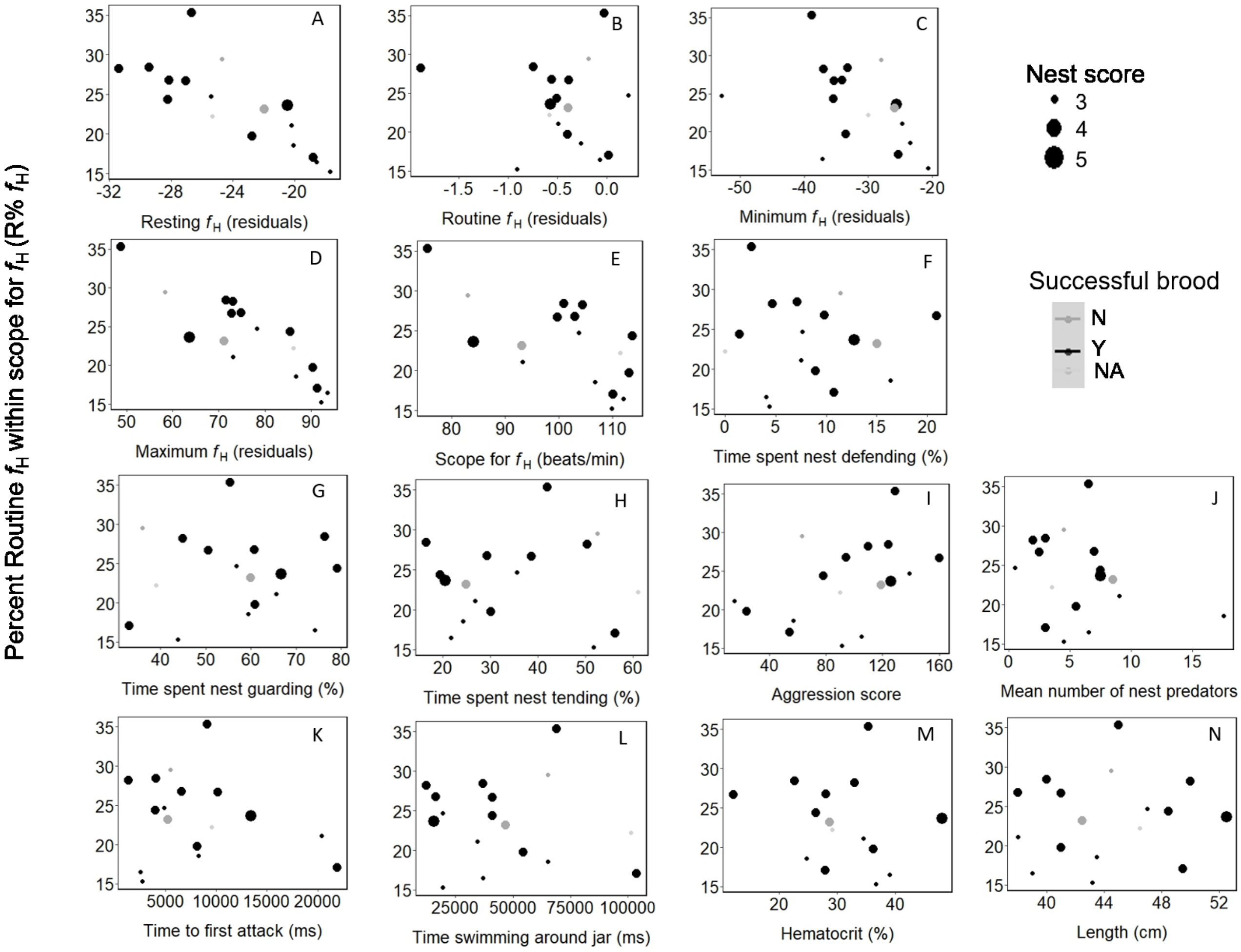

Figure 2.5 Scatterplots comparing individual percent routine f_H within scope for f_H (R%f_H), brood success, and nest score to individual (A) resting f_H; (B) routine f_H; (C) minimum f_H; (D) maximum f_H; (E) scope for f_H; (F) time spent on nest defending behaviours; (G) time spent on nest guarding behaviours; (H) time spent on nest tending behaviours; (I) aggression score; (J) nest predation pressure; (K) time to first attack; (L) time spent swimming around the jar; (M) hematocrit; and (N) Length. N =17 for all panels except M, where N = 15. Dot size represents the nest score.

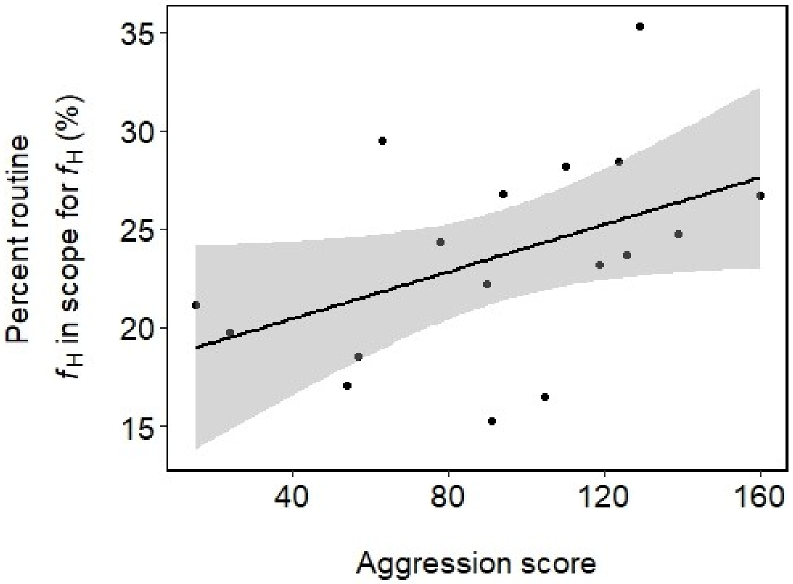

Figure 2.6 Regression plot showing the relationship between percent routine f_H within scope for f_H (R%f_H) and parental male smallmouth bass aggression score (p-value = 0.0066). Points represent individual fish responses, N=17, and the grey shaded region represents the 95% confidence region.

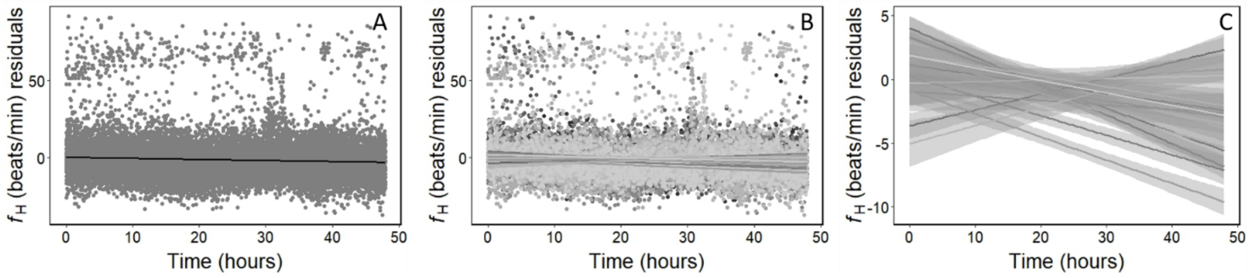

Figure 2.7 Linear mixed effect model showing parental male smallmouth bass temperature detrended f_{H} overtime where A) individual fish are pooled, B) raw data and slopes for each individual parental male bass, and C) trends of each individual parental male bass. Individuals are represented by different shades. f_{H} was recorded every 2 minutes over a 48 hours period, at 100Hz.

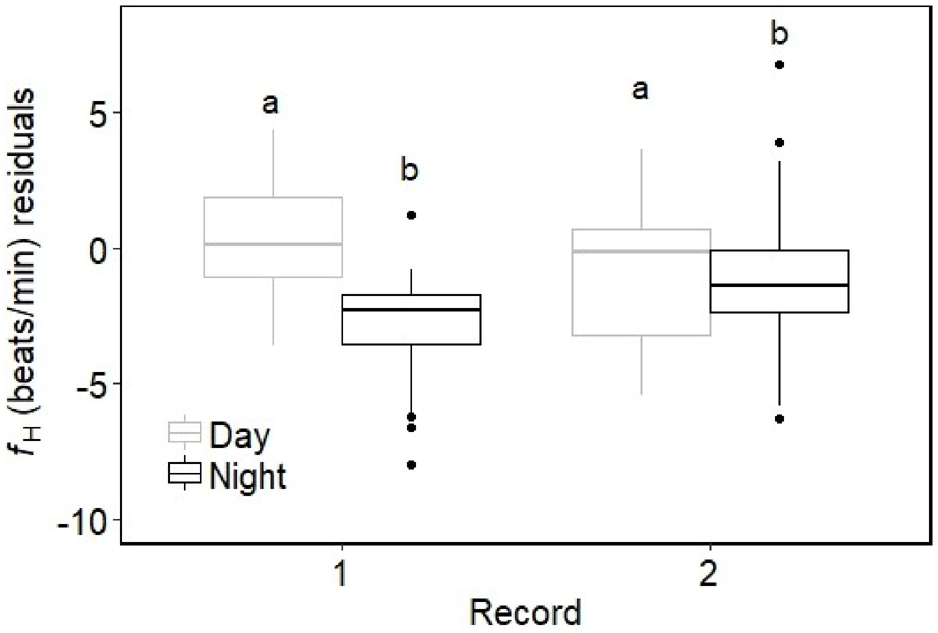

Figure 2.8 Boxplot showing the diurnal average, upper quartile, lower quartile, and standard error of nesting smallmouth bass temperature detrended percent routine f_H within scope for f_H (R%f_H) over a 48 hour period (two days and two nights). Grey represents R%f_H during the day, black represent R%f_H during night. Different letters show significant differences between groups (p-value = 0.0032).

CHAPTER 3: Cardiac performance of free-swimming wild sockeye salmon during the reproductive period

Abstract

During reproduction, animals often compete for dominance to enhance their reproductive performance. Researchers have rationalized that the ability to obtain dominance is related to the individual's physiological performance, in terms of sequestering the energy required for reproduction behaviours, and development of secondary sexual characteristics. However, studies testing this hypothesis are limited. Using sockeye salmon (*Oncorhynchus nerka*) as a model species, the present study aimed to explore the relationship between heart rate and dominance behaviour during spawning. Previous research has shown that individuals can change status on the spawning ground over time (i.e., can shift between aggregation, subordinate, and dominant). We hypothesized that individuals routinely functioning low within their scope for heart rate would be more likely to be dominant on the spawning ground, compared to individuals functioning high within their scope for heart rate. Our findings suggest that routine heart rate did not differ between males and females, yet rank order repeatability was found to be consistent in males but not in females. Furthermore, heart rate was shown to follow a diel variation, where it was overall 7% higher during the day than at night. This diel pattern became less pronounced as the spawning period progressed, where daily heart rate decreased overtime, while nightly heart rate increased. Average heart rate of the population did not differ between behavioural status (i.e., aggregation, subordinate, dominant). Additionally, at the individual level, heart rate did not change between shifts from one behavioural status to another (e.g. heart during dominance versus heart rate during aggregation). No further trends could be explained by routine heart rate, sex, other secondary sexual characteristics, survival duration or spawning success (measured in females only). Therefore, the heart rate in the present study was not related to behavioural status on the spawning ground or secondary sexual characteristics. Our study has revealed the complexity of the relationships between cardiac performance and reproductive behaviours in wild fish, but demonstrates the importance of considering environmental factors when exploring individual performance variation in future studies.

Introduction

During reproduction, dominance is a common strategy used to enhance reproductive success (discussed in Ellis 1995). Dominance occurs when an individual in the population has a characteristic or a resource that provides greater reproduction opportunities than another individual, resulting in asymmetric reproduction between individuals in the population (Huntingford and Turner 1987; Reeve et al. 1998). It has been hypothesized that attaining such characteristics or resources is related to an individual's physiological performance (see Sloman and Armstrong 2002; Perry et al. 2004). However, most research has focused on relating reproductive behaviour with morphological variables, such as size (Quinn and Foote 1994) and secondary sexual characteristics (Shine 1979; Clutton-Brock 2009), or long-acting sex hormones (Brantley et al. 1993; Oliveira et al. 2001; Kuerthy et al. 2016). Therefore, research validating the role of real-time variation in physiological performance on dominance behaviour during reproduction is limited. This is in part because assessing the physiology of free-living animals is inherently challenging (Costa and Sinervo 2004; Spicer and Gaston 2009) but there are an increasing number of electronic tagging tools that enable researchers to do so (reviewed in Cooke et al. 2004c). Physiology and behaviour are inherently linked (Gilmour et al. 2005; Cooke et al. 2013) and are both important considerations for understanding the mechanisms behind ecological processes and organismal fitness. Assessing the relationships between intraspecific physiological performance improves our understanding of natural selection of physiological mechanisms associated with an individual's reproductive behaviour, such as dominance, subordinance, or determining when an animal is ready to start competing. As such, exploring this link is particularly important for understanding the evolutionary ecology of organisms, and how this relates to changing environments (McNamara and Houston 1996; Pörtner and Farrell 2008).

Pacific salmon *(Oncorhynchus* sp.) are a semelparous, anadromous species that migrate to spawn, cease feeding throughout this process and die afterwards. This means they have one opportunity to generate lifetime success. Pacific salmon spawn in high densities, where they compete for territory and mates, and competitive behaviour on the spawning ground has been well documented (Healey et al. 2003; Quinn 2005; Esteve 2005). Males compete to mate with several females, while females compete to find the best nest site and quality males (De Gaudemar 1998; Quinn 2005). The competitive behaviours used by both sexes include *charging*, where a fish swims up to another fish and, in some cases bites or rams its nose into the side of its conspecific, and *chasing*, where one fish continues to charge another fish that is retreating (Healey et al. 2003; Esteve 2005). Males also use *posture* displays, where the nose is pointed upwards and the dorsal fin is erect, and *lateral display* where the body is tensed and fins are spread as a possible warning to other approaching males (describe by Healey et al. 2003). As a female approaches oviposition, the dominant male must deter the subordinate males (satellite and sneaker males) that become increasingly active (Esteve 2005). After spawning, a female either builds another nest (known as a redd) to spawn again, or defends her first redd. Males then assume a subordinate role, but continue to compete for nesting females, either as subordinate or by regaining dominant status. Hence, subordinate males and females are those that have either already spawned or have yet to spawn, but do not have a redd, and are trying to dominate over one or attempting to sneak a spawning opportunity from a dominant conspecific (males only). Thus, an individual’s dominance can change considerably during the spawning period. Furthermore, given that Pacific salmon cease feeding throughout their river migration, an individual’s physiological condition (e.g. energy) is expected to affect its ability to reproduce (Alonzo and Warner 2000; Brownscombe et al. 2017). Previous research that have focused on the caloric consumption during this period have shown that sockeye salmon use half of their total energy during reproduction (Brett 1983; Hendry and Berg 1999; Crossin et al. 2004). A study using electromyogram (EMG) telemetry records suggested that, while digging was the most energy demanding behaviour for females, and posture display was the most instantaneous energy demanding behaviour for males, holding was a more energy demanding behaviour because they spent the vast majority of their time holding (Healey et al. 2003). Regardless, spawning individuals invested roughly three times more energy per day while on the spawning ground than non-spawning individuals. The high cost of dominance and spawning behaviour is likely due to the extra energetic costs of aggression, redd construction, courtship, and quivering (Webb and Hawkins 1989). Results from this study support the notion that more energy-demanding spawning behaviours require higher physiological performance but, as a trade-off, contribute to the individual's reproductive fitness. However, a direct assessment of this relationship has yet to be made.

The cardiovascular system is a logical biological unit to assess physiological performance given that it is responsible for distributing oxygen, nutrients, hormones and cellular waste (reviewed in Hoar et al. 1992; Pörtner and Farrell 2008). Originally, heart rate (f_H) was not considered to be an effective proxy for performance, specifically energy expenditure, because exercising fish can alter cardiac output by regulating stroke volume independently of f_H (Farrell et al. 1984). However, recent studies have since suggested a strong relationship between metabolic oxygen consumption (i.e. energy expenditure using gross somatic energy analysis) and f_H (Clark et al. 2010). As such, variations in f_H has been successfully used in many salmonid studies to determine the physiological effects of temperature (Steinhausen et al. 2008; Eliason et al. 2011), fisheries interactions (Raby et al. 2015; Prystay et al. 2017), and feeding (Eliason et al.

2008; reviewed in Farrell et al. 2009). Furthermore, Eliason et al. (2013) demonstrated that energy acquisition and tolerance to stress is limited by the cardiovascular system in salmonids. This supports the hypothesis that f_H would be related to behaviour during spawning and reproductive performance, given the energetic pressures associated with the development of secondary sexual characteristics (Hendry and Berg 1999) and dominant behaviours (Healey et al. 2003). Another study investigating sex-specific differences in sockeye salmon physiological performance during spawning discovered that females entering spawning areas have a 21% higher routine f_H than males, likely due to costs associated with sexual maturation (e.g. gonadal development; Sandblom et al. 2009). Energy costs of reproduction behaviours have been assessed against several parameters: stored fat and protein content (Hendry and Berg 1999), cardiac performance (Lucas et al. 1993), the costs of reproduction behaviours to temperature (Steinhausen et al. 2008; Eliason et al. 2011) and sex (Clark et al. 2009; Sandbolm et al. 2009). Therefore, assessing the relationship between f_H and reproductive behaviour at an individual level, in wild, free-swimming fish is warranted.

This study aimed to evaluate the relationship between f_H and reproductive behaviour at an individual level, using f_H biologgers. Sockeye salmon (*Oncorhynchus nerka*) were selected as a model species due to the abundant research already conducted on this species. This study took place at the Gates spawning channel, D'Arcy, British Columbia, on the N'aquatqua First Nations land (50.5481°N, 122.4832°W). The spawning channel is narrow (8m wide), shallow (~0.5m deep), and just under 2 km long permitting individual fish to be easily identified and monitored. We hypothesized that if physiological performance is related to reproductive behaviour, then dominant status during reproduction will be positively related to f_H; since attaining and maintaining dominance is energy demanding, and oxygen required to create ATP (energy) is driven by the cardiovascular system (Pörtner and Farrell 2008). We therefore predicted that sockeye salmon that routinely function low within their scope for f_H would be more dominant as they would have a greater capacity to modulate their f_H during dominant behaviours, such as defending a redd. In contrast, fish that are routinely functioning high within their scope for f_H would have reduced room to modulate f_H to support activities associates with dominant status, would utilize energy reserves faster and therefore struggle to maintain dominance over a redd, have a shorter period on the spawning ground and, therefore have less opportunity to spawn.

Methods

Data collection

From Aug 24 to Sept 12, 2016, 64 sockeye salmon were individually dip netted form the entrance to the Gates spawning channel. Sockeye salmon were placed into a trough with a pump continuously replacing the water in the trough with water from the channel. Fish were electro-sedated using fish handling gloves (Smith-Root, Inc., Washington, USA, http://www.smith-root.com; 25mA) and a 2 mL blood sample was collected using a 21-gauge needle and a heparinized vacutainer (lithium heparin, 3 mL; BD, Franklin Lakes, NJ 07417, USA) to measure hematocrit. Fish were then equipped with commercially available f_H loggers (DST milli HRT, 13mm x 39.5mm, Star-Oddi, Iceland; http://www.star-oddi.com/) programmed to record f_H every 5 min at 100 Hz, and raw electrocardiogram (ECG) traces every 1.5 h to validate the quality of the f_H measurements. f_H loggers were implanted via a 3 to 5 cm incision between the pectoral fins, inserted immediately posterior to the pericardial membrane, and anchored to the ventral musculature (PDS II polydioxanone suture; violet monofilament, 3-0). The incision was then closed using 4 to 5 square knot sutures.

Secondary sexual characteristics and fish condition were also recorded. This included recording the fork length, depth, circumference, kype length (eye to nose), percentage of fat (using a handheld microwave fat meter (Distell Fish Fatmeter FM 962, Distell.com Inc., West Lothian, UK)), colour (qualitative score from 0 to 3, where 0 = silver; 1 = dark silver with some red; 2 = light red; 3 = red), and injury score (qualitative score from 0 to 3, where 0 = no injuries or fungus; 1 = minor scratches; 2 = scratches and fungus; 3 = portion of tissue missing and fungus – a similar system has been used in previous studies e.g. Raby et al. 2015). Sockeye salmon were then externally tagged with a spaghetti tag behind the dorsal fin and a Peterson disk tag on either side of their dorsal musculature, immediately anterior to the dorsal fin. The Peterson disks were labelled with a unique number to identify between individual fish, as well as colour coded by sex. Finally, the rough weight of the sockeye salmon was obtained by placing the fish in a rubber holding bag suspended from a spring-loaded scale. At this point, the fish were no longer sedated. Fish were then released into the channel where they swam to low-flow water. Hematocrit was determined using heparinized capillary tubes (75 mm Drummond Hemato-Clad, ammonium heparin) centrifuged at 8000 g for 5 min.

The day after release, individual sockeye salmon behaviour in the spawning channel was monitored daily. Fish were identified from the channel banks using binoculars, while ensuring not to scare the fish. Behaviour assessments lasted roughly 3 minutes, enough to assign each individual to one of three observable behaviours: aggregating, dominant, or subordinate (Healey et al. 2003; Esteve 2005). Aggregation was defined as when fish were clustered and holding in pools. Dominance was defined as when fish were in a position to spawn, meaning females were on a nest, digging and chasing other fish, and males were defending a territory and outcompeting other males when challenged. Subordinate was defined as when a fish was attempting to take over the territory of another fish, sneak onto a nest with a spawning female, or simply holding alone. The behaviour and the time that behaviour occurred were recorded for each observation.

After natural mortality, the carcasses were collected and the f_H loggers removed. Depth, circumference, percentage of fat, weight (using a digital scale, Ohaus Trooper), and length were re-measured.

This study was conducted in accordance with the Canadian Council on Animal Care guidelines and in accordance with the standards set by Carleton University and University of British Columbia (licence # XR 289 2016).

Statistical analyses

A total of 64 fish were implanted with f_H loggers, however only 55 were used for analysis. Fish were removed from the sample due to predation (N=1), failed logger recovery (N=4), and logger failure (N=4). Hematocrit levels indicated that the remaining fish were in good condition, where in all cases hematocrit was > 20% (average of 36% ± 0.8%; Gallaugher and Farrell, 1998).

Temperature (recorded by the f_H biologgers) varied throughout the study period, ranging between 8-15 °C, where not all individuals were exposed to the same temperature. A simple linear regression suggested temperature had a positive effect on f_H. Therefore, temperature was corrected against by determining the linear regression between f_H and temperature (rounded to the nearest 1 °C), and using the f_H residuals for temperature detrended f_H values when specified. One-way ANOVAs were used to compare routine f_H (average of f_H trace), minimum f_H, resting f_H (average of the lowest 10%), maximum f_H (average of the highest 5 percent), scope for f_H (difference between maximum and resting f_H), and the percent of routine f_H within the scope for f_H (R%f_H; calculated as ((routine-resting) ÷ scope for f_H) × 100) between the two sexes.

To determine whether variability in f_H differed between sexes, the intraclass correlation coefficient (ICC) was calculated from the linear mixed effect model comparing f_H between sexes, with individual fish as a random effect to account for repeated measures (*nlme* package; Pinheiro et al. 2014). A second linear mixed effect model with f_H residuals (temperature detrended) as the dependent variable, time, cycle (day or night) and sex as the independent variables, and individual as a random effect, was used to determine whether f_H followed a diel pattern. Model selection was conducted using stepwise model simplification (drop1 command in RStudio v. 3.2.3, RStudio Inc., Boston, MA, USA; https://www.rstudio.com/). This process was repeated twice, once with random slopes, once with both to see variation in f_H between and within individuals as time on the spawning ground progressed. However, the same results were obtained using the linear mixed effects model with random intercepts, random slopes, and with random slopes and random intercepts, but the AICc was lowest in the random intercepts model (random intercept AICc = 4856; random slope AICc = 4860; random slope and random intercept = 4860), suggesting that the random intercepts model fit the data best. Next, individual rank order repeatability in f_H during the day and during the night was determined using Spearman's rank correlation for each sex. Another linear mixed effect model was used to compare f_H residuals over time with cycle, sex, secondary sexual characteristics, and fish density on the spawning ground, and with individual as a random effect. To avoid overfitting the models, random forest analysis (*random forest* package; Liaw and Wiener 2015) was used to select the top three covariates according to %IncMSE. In this case condition factor ($K = 110 \times (\text{weight} \div \text{length}^3)$), weight, and injury were selected, where the percent variance explained was -34.24%. Weight was correlated with kype length, body depth, and body circumference and was therefore the only variable of the four included in the random forest analysis. The linear mixed effect model was further simplified using stepwise model simplification. To determine whether f_H at night influenced the f_H of the subsequent day, the same process was repeated using f_H (residuals) during the day as the response variable and f_H (residuals) during the night as the independent variable. Again, random forest analysis suggested K, weight, and injury to be the most influential covariates (percent variance explained = -31.81%). A fourth linear mixed effect model was used to relate f_H at night to sex, injury, colour, and density (random forest = -28.66).

Given that R%f_H is a continuous variable bound between 0 and 1, a beta regression model (*betareg* package, Zeileis et al. 2016) was used to determine whether R%f_H related to secondary sexual characteristics and survival duration on the spawning ground (Kieschnick and McCullough 2003). Random forest analysis was used to select the top three covariates, survival, fat content, and colour content (percent variance explained = -14.85%), and Akaike information criterion (AICc) was applied for further model simplification (full model AICc = -95.61; simplified model with only survival as a covariate, AICc = -126.68). This process was repeated to relate R%f_H to the gonadal somatic index (GSI; calculated as (gonad wet weight (g) ÷ fish wet weight (g)) × 100) as a metric of spawning success in females (full model included GSI, fat content, and colour, random forest percent variance explained = -8.37, AICc = -50.31; simplified model = -66.13).

f_H associated with each spawning behaviour was determined by taking the average f_H during the 15 minutes around where behaviour was recoded (five minutes before, during and after). A linear mixed effect model was used to relate average overall f_H and R%f_H between the three behaviours, with individual as a random effect. This model was then repeated to examine whether within individual differences in f_H between behaviours could be related to the type of behavioural shift (i.e., change in f_H from subordinate to dominant vs change in f_H from dominant to aggregation). In this case f_H was not temperature detrended, since changes in f_H were being quantified within the individual, where temperature hardly varied (average change in temperature with behaviour shifts = 0.015°C ± 1.05°C). Instead, average temperature and differences in temperature between behaviour shifts were included as covariates. All models were repeated to determine whether variation in f_H could be further related to sex, secondary sexual characteristics, survival, or density on the spawning ground (random forest analysis percent variance explained < 17% for all cases), and GSI in females. Models were simplified using stepwise model simplification (drop1 command) when appropriate. Finally, cox proportional hazard analysis was used to determine the relationship between GSI and survival duration in females.

All statistical analyses were conducted in RStudio. Models were tested at a 95% confidence level ($\alpha = 0.05$), and all model assumptions were validated.

Results

Heart rate profile

Figure 3.1 shows examples f_H traces from a male, a female that did not spawn, and a female that did spawn. Overall maximum (78.1 ± 1.9 beats min^{-1}), minimum (14.3 ± 0.38 beats min^{-1}), resting (26.6 ± 0.91 beats min^{-1}), routine (47.7 ± 1.1 beats min^{-1}), and scope for f_H (51.5 ± 1.8 beats min^{-1}) did not differ between sexes (all p-values > 0.1; Figure 3.2). Although this was measured when temperature had a positive effect on f_H ($r^2 = 0.92$; p-value < 0.01; Figure 3.3), where f_H increased by 2.7 ± 2.1% per 1°C increase, no difference in f_H parameters between sexes remained even after f_H was temperature corrected (p-value > 0.1 for all cases).

Average R%f_H was 40.87 ± 1.0%, and did not differ between sexes (p-value > 0.1). Neither did R%f_H relate to survival (p-value = 0.10) or secondary sexual characteristics (p-value

> 0.1 for all) for either sex. Additionally, variability in f_H did not differ between sexes (ICC = 0.11). For both sexes, f_H followed a diel pattern where f_H during the day was 7 ± 0.4% higher than f_H at night (model outputs presented in Table 3.1; Figure 3.4). The linear mixed effects model relating f_H with time suggested that, despite the variability between individuals (Table 3.1), f_H during the day (day starting at 6:30; night starting at 20:00) decreased over the spawning period while f_H at night increased (Figure 3.5). Although the change in f_H over the course of the study did not significantly differ between sexes (removed from the model during model simplification; Table 3.1), the diel trend appears to be stronger in males than in females given the steeper slopes (Figure 3.5). The change in f_H over time could not be explained by secondary sexual characteristics, survival duration, or fish density on the spawning ground (p-values > 0.1 for all cases). However, irrespective of time since arrival to the spawning ground, an individual's f_H at night was positively related to f_H of the subsequent day at the individual level (Table 3.2; Figure 3.6). This was common across both sexes, but again, there appears to be a stronger trend in males than in females given the steeper slope.

Daily f_H (average over 24 hours) was not repeatable in either sex. However, when separated by day and night, f_H repeatability was detected in the males at night (p-value <0.001) and was marginally non-significant during the day (p-value = 0.072). There was no repeatability detected in the females (p-value > 0.1 for all cases).

Relationship between heart rate and behaviour

On average, fish were observed for 7.21 ± 0.4 days before they died. During this period, individuals were observed in aggregation for 1.69 ± 0.2 days, subordinate for 3.4 ± 0.3 days, and dominant for 2.16 ± 0.1 days (Figure 3.7). After pooling the average f_H of all fish, f_H did not differ among the three behavioural statuses (p-values = 0.59; Figure 3.8). Similarly, there was no effect of sex, secondary sexual characteristics, or fish density on f_H during each behavioural status (all cases p-values > 0.1). Also, when individual changes in f_H were analyzed, there was no significant differences in f_H between types of behaviour shifts (p-value > 0.1; Figure 3.9). The largest change occurred when fish shifted from subordinate to dominant, where the f_H increase by 4.5 ± 1.1 beats min^{-1}, but this was not significantly different from other shifts (n=29; p-value > 0.1 compared to other behavioural shifts). Over the limited time of monitoring period, an individual was rarely dominant for more than one cycle (Figure 3.7). As the dominant fish retreated, another fish became dominant.

Individual changes in f_H could not be explained by the individual's overall f_H parameters (i.e. R%f_H, minimum f_H, maximum f_H, resting f_H, or scope for f_H; all p-values > 0.1 – in models using random intercepts, random slopes, and random intercepts and slopes). Similarly, the changes in f_H between behaviour status did not relate to secondary sexual characteristics or sex (all p-values > 0.1).

Relationship between heart rate, behaviour and GSI (females only)

Neither R%f_H nor changes in f_H between behaviours were related to an individual's GSI after death (p-values > 0.1 for both cases). Additionally, survival duration on the spawning ground did not change with GSI (hazards ratio = 0.93, z = -0.97, p-value = 0.92; Figure 3.12), where on average both groups survived 7.4 ± 0.7 days on the spawning ground (males = 7.17 ± 0.2 days).

Discussion

The present study aimed to test the hypothesis that physiological performance is related to reproductive investment (Franklin and Davie 1992; Sloman and Armstrong 2002; Ricklefs and Wikelski 2002; Perry et al. 2004; Clark et al. 2013) by exploring the relationship between f_H

performance and dominance behaviour in spawning sockeye salmon. Our study did not detect any differences in f_H between different types of reproductive behaviours, sex, secondary sexual characteristics, spawning status, or fish density on the spawning ground. Thus, the present results do not support the hypothesis that individuals need to be capable of adjusting their f_H (i.e. physiological performance) to attain a spawning status. This is the first study, to our knowledge, to explore the relationship between individual level f_H and behaviour across several reproductive phases in spawning fish. However, our study is among multiple other studies failing to demonstrate that physiological performance is related to reproductive investment in fish (e.g. Wiegmann and Baylis 1995; Hatfield and Schluter 1999; Hanson et al. 2009).

Characterization of spawning sockeye salmon heart rate

The f_H recorded in this study were comparable to the f_H recorded in previous studies on adult sockeye salmon (Table 3.3). Sockeye salmon routine f_H in the present study was on average 40 ± 1.0% of their scope for f_H, irrespective of sex. Relatively small differences between the values obtained in this study and values in the literature could be due to different sockeye salmon populations used in the study (e.g., Quesnel vs Early Stuart vs Gates), where populations have unique physiological adaptations related to thermal environment and level of migratory difficulty (Eliason et al. 2011). Another reason for differences in recorded f_H could be due to the fact that, with the exception of Clark et al. 2009, previous studies that have focused on spawning-phase fish restricted individuals to an enclosure (Sandblom et al. 2009; Clark et al. 2010), whereas fish in the present study were free-swimming throughout the spawning channel. Nevertheless, the present study did require surgical procedures and fish handling, which typically requires hours-to-days for full recovery (Raby et al. 2015; Prystay et al. 2017). Although fish were given 24 hours to recover (restricted due to the nature of the project), the surgery could have still influenced reported f_H values.

Previous research has recorded spawning female sockeye salmon f_H to be 21% higher in females than males upon entering the spawning ground (Sandblom et al. 2009), possibly due to a greater oxygen demand driven by the larger female gonads, however this has yet to be quantified (Clark et al. 2009). In contrast, spawning males have been shown to spend 15% more time at f_H above 50 beats min^{-1} than female sockeye salmon (although this result was non-significant), but routine oxygen consumption did not differ between sexes (Clark et al. 2009). Neither direction was favoured in the present study given there was no difference detected in overall f_H between sexes (p-value > 0.1). This could be due to the comparatively longer sampling period (< 1 day in the Sandblom et al. (2009) study vs. an average of 7 days in the present study), larger sample size in the present study (11 and 13 individuals in the Clark et al. (2009) and Sandblom et al. (2009) study, respectively vs. 55 individuals in the present study), and different sampling frequencies (i.e. Clark et al. 2009 recoded f_H continuously, whereas the present study recorded f_H for 6 seconds every 5 minutes). Regardless, it has been well documented that mature female sockeye salmon experience a higher mortality rate in response to stressors than males (Martins et al. 2012; Burnett et al. 2014), and have higher cortisol concentrations than males (Kubokawa et al. 1999; Sandblom et al. 2009; Hruska et al. 2010). It therefore makes sense that females would have a higher f_H since the endocrine and cardiovascular systems are linked (Farrell et al. 1988). Alternatively, previous research has shown that, although the caloric breakdown of behaviours results in females burning on average 1,109 more calories per day than males, the difference between sexes was not significant due to large intraspecific variation (Healey at el. 2003).

Similarly, the intraspecific variability in f_H was likely masking differences in f_H between sexes in the present study (discussed further under *Heart rate and spawning behaviours*).

Environmental factors influencing heart rate

It is well known that the environment has an effect on f_H. Studies on sockeye salmon have shown that Q_{10}, the factorial change in f_H with every change in 10°C, is ~2 (Farrell 1992; Steinhausen et al. 2008; Eliason et al. 2013; Eliason and Anttila 2017). The f_H of the sockeye salmon in the present study increased by 2.6 beats min^{-1} with every increase in 1°C (net Q_{10} for study period = 1.5).

After correcting for temperature, sockeye salmon f_H followed a diel pattern where f_H was 7% higher during the day that at night. Diel variation in f_H has been documented in teleost species (Pickering and Pottinger 1983) including other studies conducted on spawning sockeye salmon, where diel patterns in f_H mimicked diel variation in visceral temperature (Clark et al. 2009; 2010). However diel variation was not detected in previously recorded spawning sockeye salmon electromyogram (EMG) records (Healey et al. 2003), suggesting behaviour does not follow a diel pattern. Such variation in f_H is therefore likely driven by photoperiod, where time of day stimulates changes in other physiological parameters, such as hormones and blood plasma constituents, that were not included in the present study but can potentially drive changes in metabolic rate (Hoar et al. 1992; Farrell 1993; Vornanen 2017). Furthermore, the difference between day and night f_H decreased over time as the spawning period progressed (Figure 3.5). A potential explanation for this trend is that circadian rhythms become more relaxed as fish approach senescence. Hruska et al. (2010) demonstrated that as sockeye salmon senesce, hormone and metabolite levels fluctuate, where lactate and cortisol increase, and $[Na^+]$, $[Cl^-]$ and osmolality decrease, thus supporting the notion that fish undergo physiological stress as they approach senescence. The intrinsic link between the cardiovascular system and the endocrine system suggests that such changes in hormone and metabolites can further influence f_H (e.g. f_H increases with increasing cortisol; Sandblom et al. 2009, discussed in Hoar et al. 1992 and Farrell 1993; Vornanen 2017). Additionally, gross somatic energy reserves, including glucose levels decrease (Rodnick and Gesser 2017), implying there is less available energy to maintain the frequent muscle contractions required for elevated f_H (Hruska et al. 2010). Therefore, it is possible that overall maximal routine f_H (i.e. day) decreases, while f_H at night must increase to maintain sufficient cardiac output for survival (according to the equation that cardiac output = stroke volume × f_H; Priede and Tytler 1997). Lastly, the slower rate of change in f_H observed in females (indicated by the smaller slopes) may be due to differences in plasma hormones and metabolites compared to males (e.g. higher cortisol), and possibly because females have a higher hemoglobin concentration than the males, increasing the female's blood-oxygen carrying capacity (Clark et al 2009).

In addition to temperature and diel variation, previous studies have shown that density of individuals would likely have an effect on behaviour and therefore, physiological performance (Montero et al. 1999; Spence and Smith 2005). In the case of the present study, fish density on the spawning ground was not correlated to f_H nor behaviour (see *Heart rate and spawning behaviours*). This is probably because the present study was conducted in an artificial spawning channel, where the number of fish entering the spawning channel was controlled, and fish were able to distribute themselves within the spawning channel reducing competition. This could also be due the poor record of the actual fish density on the spawning channel, given that the electronic counter used to assess fish numbers was malfunctioning during the time of this study. Thus, we had to use estimates from the electronic counter data which could have been erroneous.

Future research is required to further investigate the relationship between f_H and density of spawning sockeye salmon.

Lastly, predation pressure is another environmental factor that has been shown to affect f_H in previous literature (Johnsson et al. 2001; Donaldson et al. 2010). However, this study was conducted in a controlled spawning channel, where redd predators were kept out. With the exception of the odd bear and bird predation, the sockeye salmon were generally protected from predators during spawning. Future studies are required to investigate whether salmon spawning in areas with more natural predator burdens would show the same f_H patterns.

Heart rate and spawning behaviours

Time associated with each behavioural status during spawning varies between sockeye salmon populations (Healey et al. 2003), where in the present study spawning sockeye salmon spent more time as subordinate and the least amount of time in the aggregation phase (Figure 3.7). Using EMG data to estimate the caloric consumption of locomotor activities during spawning, Healey et al. (2003) suggested that it was more energetically expensive to be dominant or subordinate rather than be engaged in an aggregation. However, converting the f_H data from the present study to metabolic oxygen consumption (MO_2), the metabolic costs of each behaviour status can be roughly estimated as 2.41 ± 0.26 mg O_2 min^{-1} kg^{-1}, 2.40 ± 0.22 mg O_2 min^{-1} kg^{-1}, 2.40 ± 0.32 mg O_2 min^{-1} kg^{-1}, yielding no difference between dominant, subordinate, and aggregation status, respectively. Other than the fact that the f_H to MO_2 conversion equation was derived using different sockeye salmon populations with different physiological tolerances and performance levels (data from Eliason et al. 2011), discrepancies in these estimates are likely because, according to the Fick principle, f_H is only one of the components driving MO_2 (Eliason et al. 2013; Farrell and Smith 2017). Other components include stroke volume (to determine cardiac output) and the arteriovenous oxygen extraction, which were not measured in the present study. Further discrepancies could also be caused by delayed peak and f_H recovery post-exercise, extending beyond the 15 minute intervals (Raby et al. 2015; Prystay et al. 2017).

The natural variability in f_H within and between individuals in the population may have masked differences in f_H between behaviour status'. One possible explanation is that the changes in morphology (e.g. gonads) and hormones associated with senescence (Hruska et al. 2010; Rodnick and Gesser 2017), and varying environmental factors (e.g. temperature and diel variation, see *Environmental factors influencing heart rate*) are influencing individuals differently, causing various physiological (including f_H) responses between individuals. Another possible explanation is that all fish studied had a broad scope for f_H since it has been shown that aerobic scope is positively related to whether a salmon can complete a spawning migration (Farrell et al. 2008). The true maximum f_H was not measured in the present study (to avoid interfering with spawning behaviours), potentially skewing the true variation in scope for f_H between individuals in the present study. Lastly, the lack of difference in f_H between behaviours could be due to the behaviour data being too coarse. Healey et al. (2003) showed that holding on a redd was more energetically costly for females than males because they spent more time holding than males. This suggests that the duration spent at each behaviour status strongly influences the metabolic cost. However, time spent at each behaviour status was not recorded in the present study. Instead, behaviour was only monitored once a day, and occasionally an individual was not found. As such, behaviour immediately before and after the assessment were unknown, and behaviour status could have shifted soon after the behaviour was recorded. Future studies investigating the relationship between f_H and reproductive investment in spawning salmon should include accelerometer data for more refined behaviour assessments, as well as measure maximum metabolic performance and changes in hormones associated with senescence.

Irrespective of time spent at each behaviour status, shifting between behaviour status would in hypothesis result in a change in f_H at the individual level. Aggregation requires energy to hold position (Healey at al. 2003), however this often occurs in pools (Prystay personal obs.) and in packs (Esteve 2005), where there is slower moving water and a fish can seek shelter where there is less flow behind another fish. In contrast, subordinate and dominant behaviours require burst swimming, thrashing in fast flowing water (Healey et al. 2003; Esteve 2005). However, the present study showed no significant differences in changes in f_H between behaviours (Figure 3.9). These results do not coincide with the Healey et al. (2003) EMG data, where caloric expenditures for both sexes were significantly larger during dominant and subordinate status compared to the aggregation. Instead, increased metabolic demand associated with dominant and subordinate behaviours could have been met by changes in other cardiovascular parameters, such as stroke volume (Eliason et al. 2013; Farrell and Smith 2017). Additionally, lack of significant difference in f_H between behaviour status could be because all behaviours involve holding, which is energetically expensive especially given that the fish are approaching senescence (Healey et al. 2003). Burst swims associated with subordinate and dominant status occur less frequently than holding behaviours, and could have been met by short bursts of anaerobic metabolism that resulted in non-detectible changes in f_H (Wood 1991). Coupling spawning salmon f_H data with accelerometry data in future studies would provide further insight on the fine-scale relationships between f_H and reproductive behaviours.

Heart rate and survival duration

The notion that fitness is dependent on metabolic capacity for exercise was not supported in this study (Ferrell et al. 2008; Clark et al. 2011; Eliason et al. 2013; Brownscombe et al. 2017). In females, survival duration was independent of whether the female spawned (Figure 3.10), and independent of R%f_H. Although an individual's routine f_H changed over time, the rate of change in f_H was low for both day and night (slopes of approximately 1% or less per day; Figure 3.5). The lack of relationship between overall f_H and an individual's survival duration, reproduction behaviour, and an individual's spawning success or failure (although measured in females only) suggests reproductive performance is likely driven by a different factor or, more likely, a complex combination of physiological variables (e.g., hormones, blood plasma constituents, or stored protein content; Hoar et al. 1992; Farrell 1993; Hendry and Berg 1999).

Conclusion

Our study extends the findings from previous studies by exploring the relationship between f_H and reproductive behaviour at the individual level. By doing so, the present study has revealed the complexity of the relationships between cardiac performance and reproductive behaviours in wild spawning sockeye salmon. Overall trends in f_H and behaviour status were masked due to large interindividual variation in f_H. The present results therefore demonstrate the importance of considering environmental factors when exploring between and within individual variation in future physiological and bioenergetic studies. Future studies should continue investigate the relationship between physiological performance and reproductive investment to enhance our current understanding of ecological processes in changing environments.

Table 3.1 Results for the linear mixed effects model relating f_H within scope for f_H ($R\%f_H$) with time spent in the spawning channel and diel cycle, where individual fish was treated as a random effect (N = 55). Colon represents an interaction between cycle and time. Statistically significant differences are show in bold, while marginally non-significant variables are identified with an asterix (df = 698; α = 0.05). Same results were obtained in the linear mixed effects model using random slopes, however the AICc was larger in the random slope model (random intercepts AICc = 4856; random slopes AICc = 4860; random slopes and random intercepts = 4860).

	Value	**SE**	**t-value**	**p-value**
Intercept	1.7	0.52	3.2	**0.0013**
Cycle (Day vs Night)	-4.1	0.76	-5.3	**<0.0001**
Time (in channel)	-0.036	-0.048	-0.77	0.44
Cycle:Time	0.13	0.069	1.9	0.060*

*Step-wise model simplification removed sex from the model.

Table 3.2 Results for the linear mixed effects model relating heart rate (f_H) during the day with f_H from the previous night with an interaction with sex. Individual fish were treated as a random effect. Colon represents an interaction between cycle and time. Statistically significant differences are show in bold (α = 0.05).

	Value	**SE**	**DF**	**t-value**	**p-value**
Intercept	1.32	0.39	281	3.40	**<0.001**
f_H night	0.45	0.057	281	7.87	**<0.001**
Sex	0.51	0.60	51	0.86	0.39
f_H night:Sex	-0.25	0.10	281	-2.53	**0.012**

Table 3.3 Sockeye salmon heart rate (f_{H}) recorded in the present study and in previous literature.

Population	**Type**	f_{H} ***(beats min^{-1})***	**Temperature (°C)**	**n**	**Study**
Weaver	Routine	48	12	13	Sandbolm et al. 2009
	Scope	60 *(f_H ranged from 20 to 80 beats min^{-1})*	10	11	Clark et al. 2009
	Routine	~ 40	11.5	11	Clark et al. 2010
		~ 50	14		
Early Stuart	Resting	70.1 ± 2.3	15-20	9	Eliason et al. 2011; 2013
	Max	90.3 ± 3.7			
	Scope for f_{H}	~ 25			
Quesnel	Resting	60.9 ± 4.7	15-20	6	
	Max	97.7 ± 7.2			
	Scope for f_{H}	~ 40			
Chilko	Resting	67.3 ± 2.7	15-20	13	
	Max	94.0 ± 3.2			
	Scope for f_{H}	~75			
Gates	Max	78.1 ± 1.9	8-15	55	Present study
	Min	14.3 ± 0.38			
	Resting	26.6 ± 0.91			
	Routine	47.7 ± 1.1			
	Scope for f_{H}	51.5 ± 1.8			

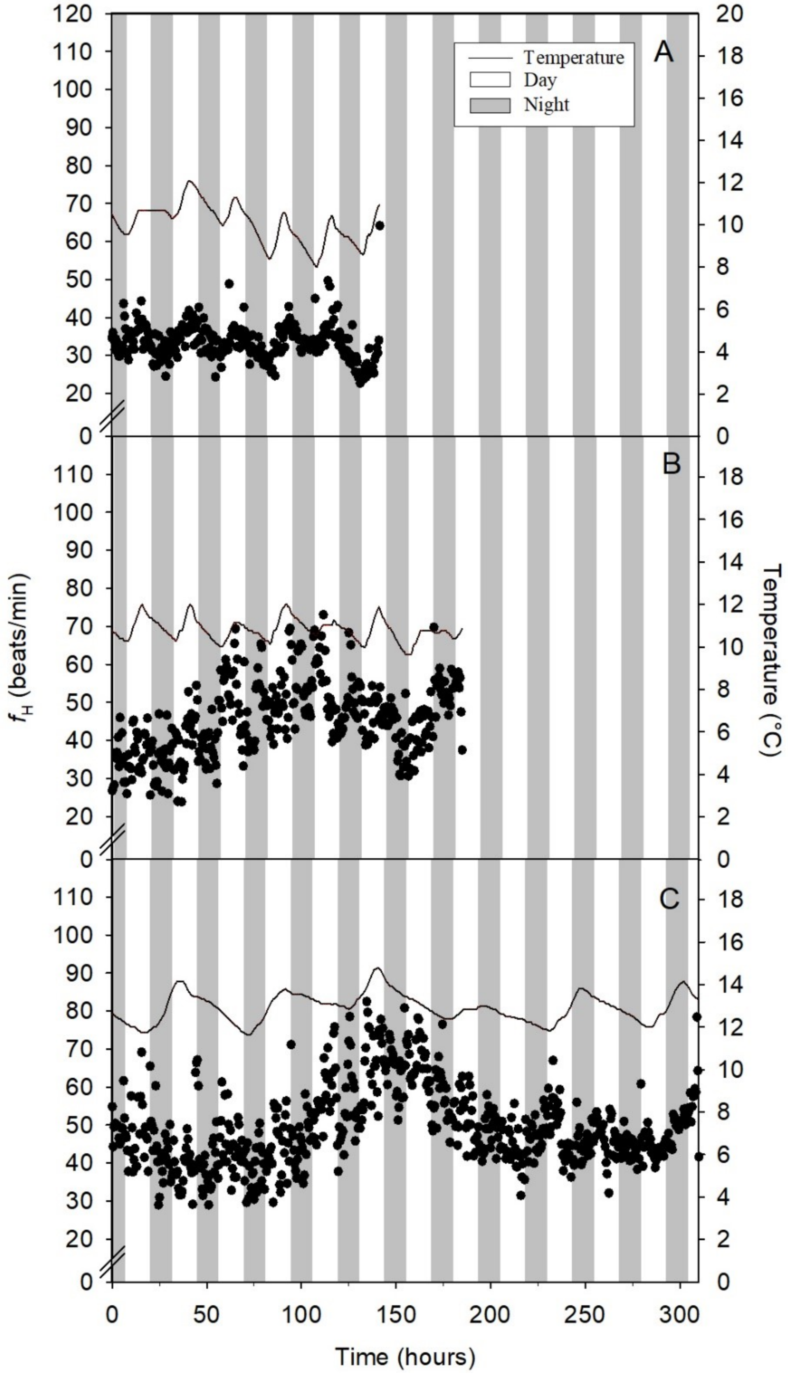

Figure 3.1 Scatterplot showing the heart rate (f_H) (beats min^{-1}) trace of an individual spawning male (A), spawning females with GSI = 9.8% and did not spawn (B), and spawning female with GSI = 0% and did spawn (C) averaged for every half hour (black dots). f_H was recorded from spawning channel entry to mortality. Dashed line is the regression line for the water temperature. White bands represent daytime (6:30) and grey bands represent night (20:00).

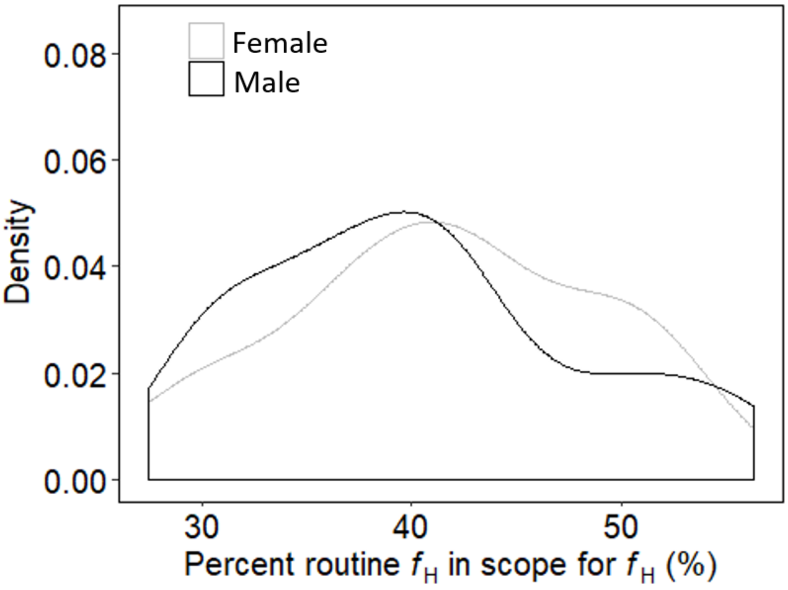

Figure 3.2 Density distribution plot showing the frequency of individual variation in routine f_H within scope for f_H (R%f_H) (%) of individual spawning male (n = 26; black) and female (n = 29; grey) sockeye salmon.

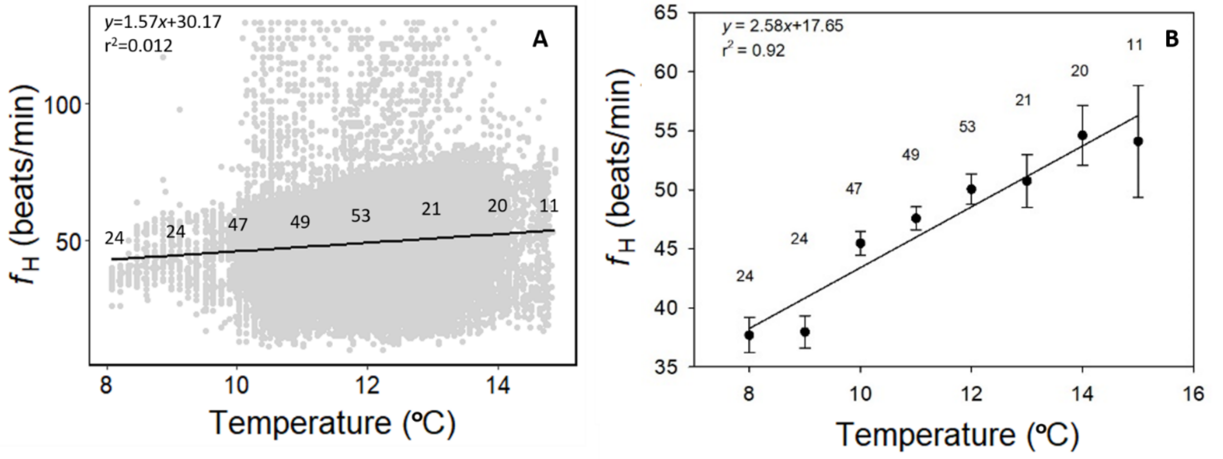

Figure 3.3 Regression plot showing A) raw and B) mean f_H of 55 spawning sockeye salmon in response to water temperature increase in 1°C increments. The regression line shows that f_H increases according to the equation $f_H = 1.57$ (temperature °C) + 30.17, with a coefficient of determination (r^2) of 0.012, p-value > 0.05, but on average f_H increases according to the equation $f_H = 2.58$ (temperature °C) + 17.65, with an r^2 of 0.92, $p < 0.001$. Error bars represent the standard error for each mean. Numbers over each point describe the number of individuals exposed to each temperature.

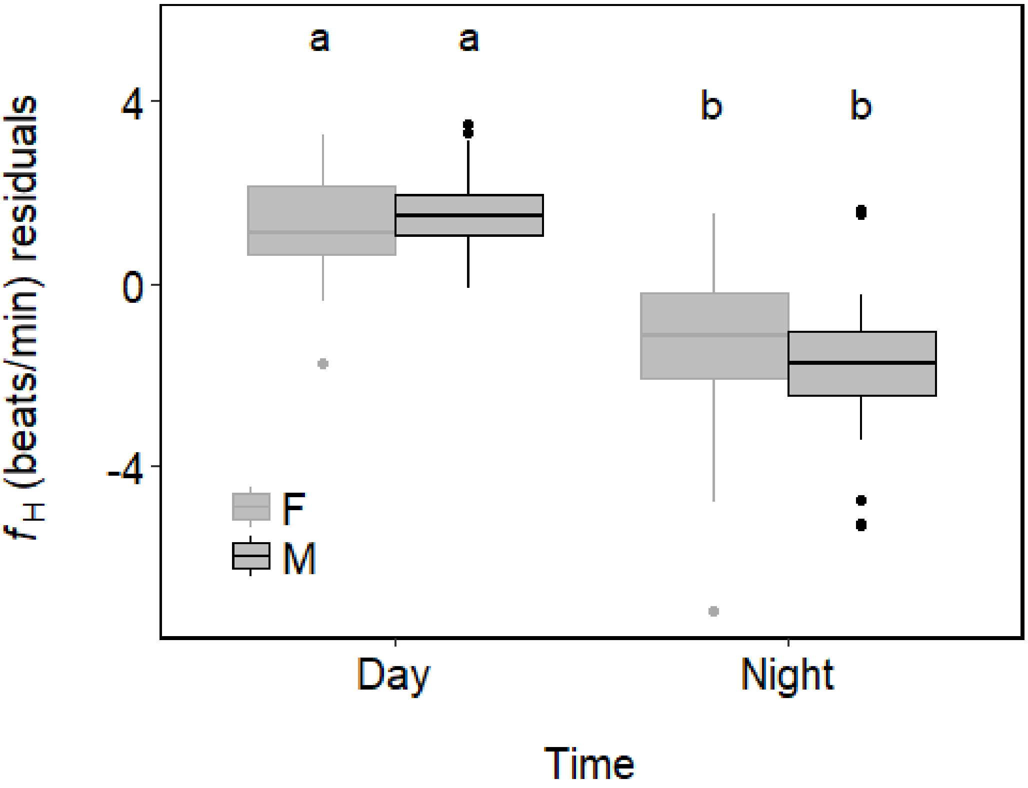

Figure 3.4 Boxplot showing the diurnal average, upper quartile, lower quartile, and standard error of spawning female (grey, n = 29) and male (black, n = 26) sockeye salmon percent routine f_H within scope for f_H (R%f_H) over a spawning period. Different letters show significant differences between groups ($p < 0.0001$).

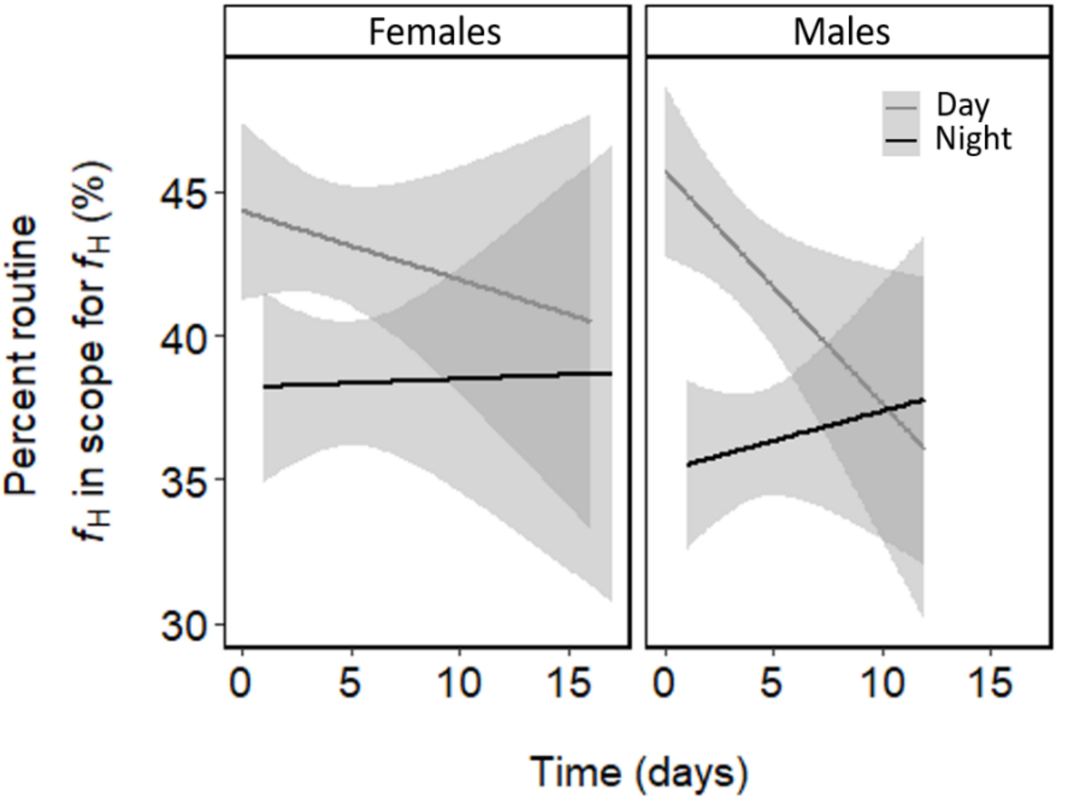

Figure 3.5 Linear mixed effect model showing the changes in diel heart rate (f_H) as percent routine within scope for f_H (R%f_H) over time in spawning male (left) and female (right) sockeye salmon. Grey line represents the f_H during the day, black line represents f_H during the night. Standard error is shown in grey.

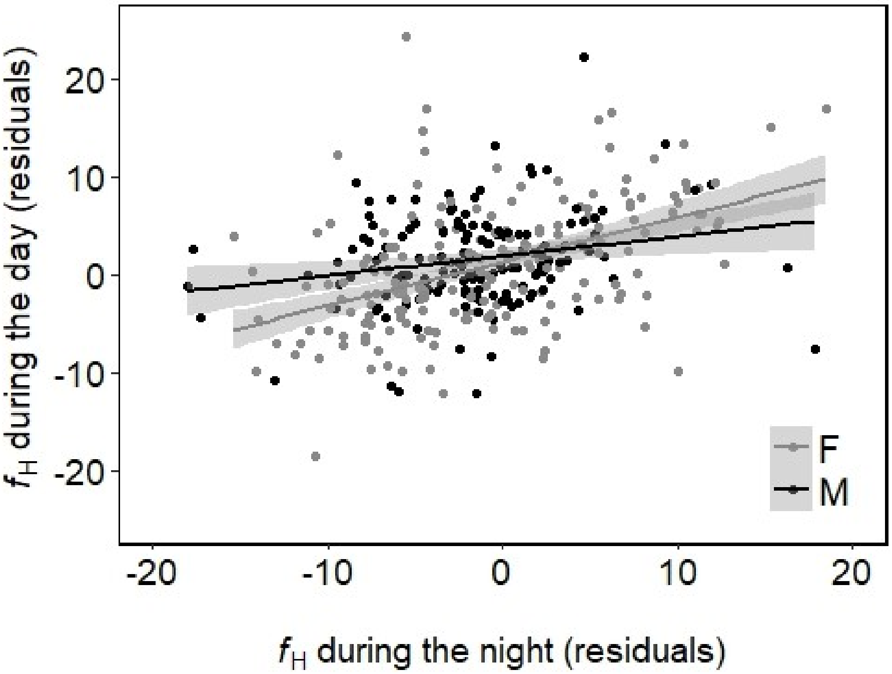

Figure 3.6 Linear mixed effect model showing the relationship between heart rate (f_H) measured at night and f_H measured the subsequent day. Grey line represents the relationship within females, black line represents relationship within males. Grey dots represent individual females, black dots represent individual males. Standard error is shown in grey.

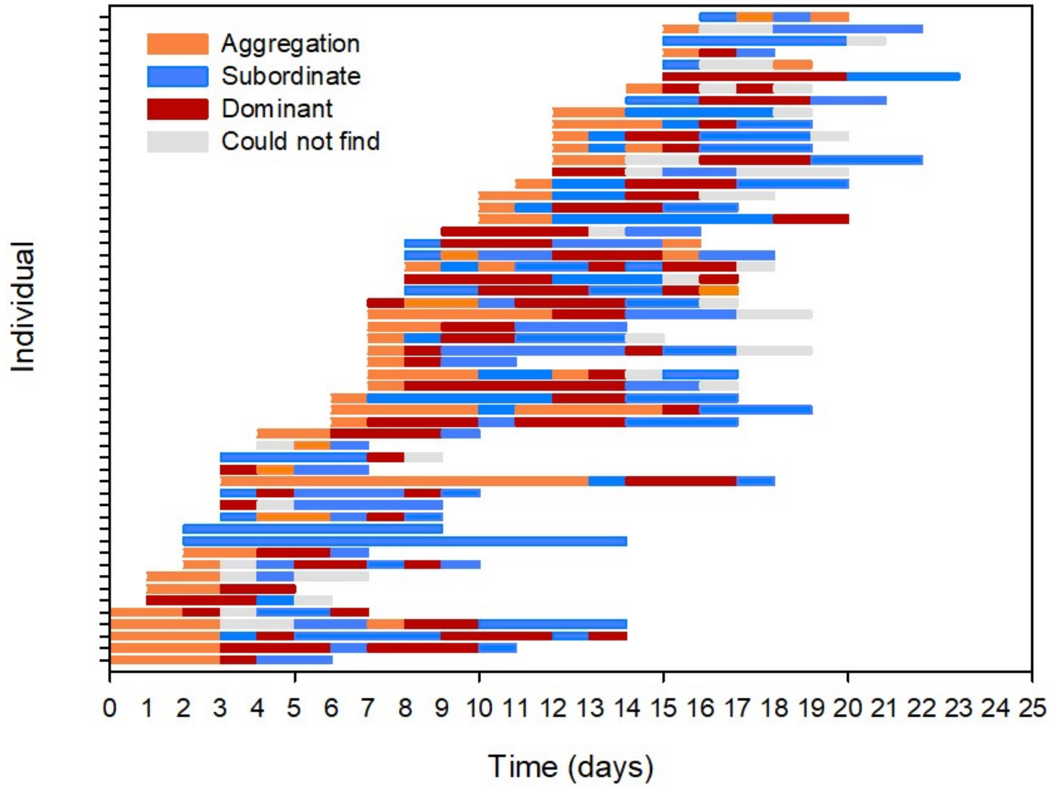

Figure 3.7 Observed time (day) spent on each spawning behaviour status. Orange represents aggregation behaviours status (fish were clustered and holding in pools), blue represents subordinate behaviour status (fish were attempting to take over the territory of another fish, sneak onto a nest with a spawning female, or simply holding alone), and red represents dominance behaviour (were in a position to spawn, meaning females were on a nest, digging and chasing other fish, and males were defending a territory and outcompeting other males when challenged). Grey represents when the fish could not be found after several passes with two observers.

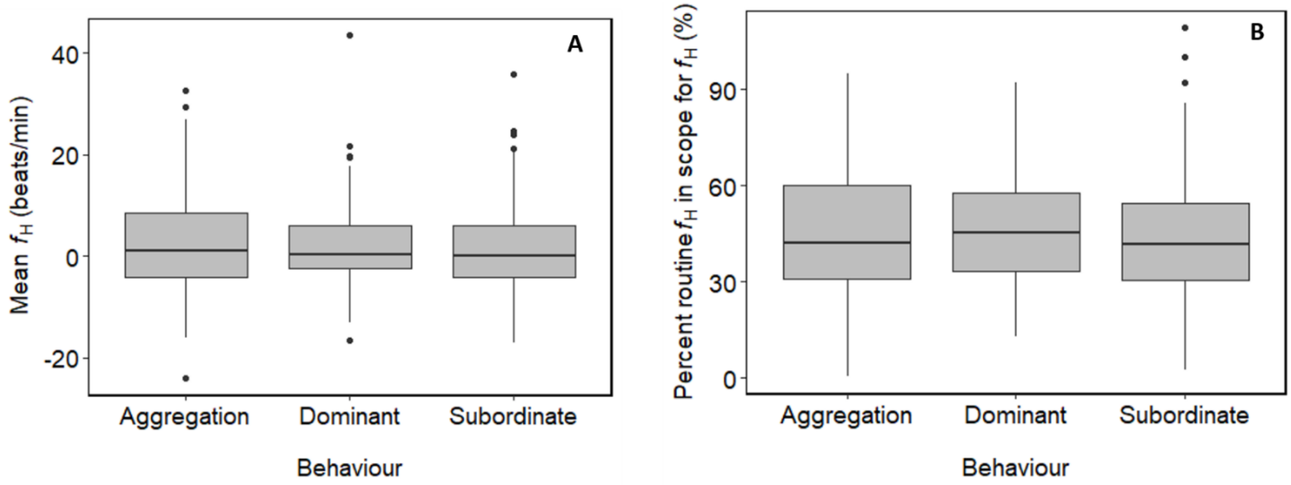

Figure 3.8 Mean heart rate (A) and routine f_H within scope for f_H (R%f_H) (B) during the three behaviour statuses (dominant, subordinate, aggregation).

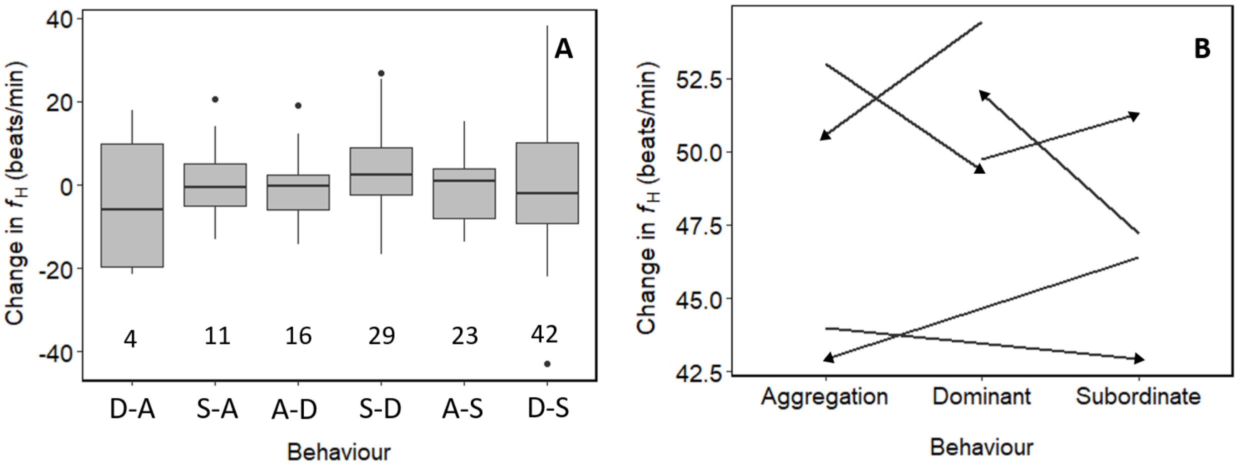

Figure 3.9 Individual variation in average changes in heart rate (f_H) within scope for f_H (R%f_H) (A) and magnitude of change in f_H (B) during shifts between the three behaviour statuses (dominant to aggregation (D-A), subordinate to aggregation (S-A), aggregation to dominant (A-D), subordinate to dominant (S-D), aggregation to subordinate (A-S), and dominant and subordinate (D-S)). Individual was treated as a random effect to account for repeated measures. Number below boxplots show the sample size for each behaviour transition.

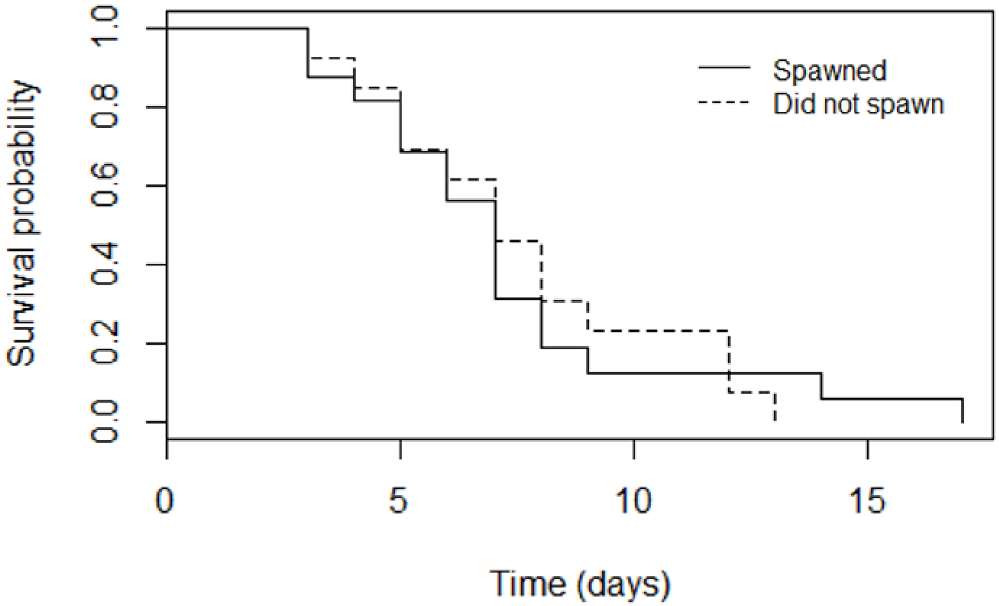

Figure 3.10 Survival probability of female sockeye salmon that spawned compared to female sockeye salmon that did not spawn according to individual's GSI after mortality, where individuals with GSI < 5% were deemed to have spawned (n = 13) and individuals with GSI > 5% were deemed to have not spawned (n= 16).

CHAPTER 4: Synthesis and Conclusions

Synthesis and Conclusions

Using heart rate (f_H) as a physiological parameter, the present thesis aimed to test the hypothesis that physiological performance mediates reproductive investment and fitness related behaviours (Ricklefs and Wikelski 2002; Westneat et al. 2011; Brownscombe et al. 2017). Smallmouth bass (*Micropterus dolomieu*) and sockeye salmon (*Oncorhynchus nerka*) were used to relate f_H to parental care and dominance status, respectively. Data from the f_H biologgers revealed intraspecific variability in f_H in both species (Figure 2.3; Figure 3.2). However, despite previous evidence that enhanced energy behaviours are important for reproduction (Hinch and Collins 1991; Cooke et al. 2002a; Healey et al. 2003), and that metabolic demands are met by cardiovascular system (Priede 1985; Ferrell 1992; Pörtner and Farrell 2008), the present thesis could not confirm that routine f_H is related to an individual's reproductive investment. Instead, it is proposed that reproductive behaviour is mediated by a combination of physiological and environmental factors, and not by and individual's routine f_H alone.

It was originally predicted that parental male smallmouth bass routinely operating low within their scope for f_H would have a greater capacity to increase their f_H to meet the enhanced metabolic demands of parental care behaviours. In contrast, individuals routinely functioning high within their scope for f_H would be limited by their physiological capacity to cope with the energy demands of reproduction behaviours. These individuals would experience elevated pressure from parental care burdens (e.g. temperature fluctuations and nest predators), and would be more likely to spend less time on nest tending parental care behaviours, abandon their nest, and hence be less reproductively fit than individuals routinely operating at a lower scope for f_H.

Instead, however, there was no detected relationship between where an individual routinely operated within their scope for f_H and time spent on different reproduction behaviours (i.e. nest tending, nest guarding, and nest defending). Despite the lack of relationship with parental care behaviours, f_H was shown to be positively related to parental male aggression level (Figure 2.6), suggesting a relationship between f_H and parental care performance may become apparent if studied in systems with a greater nest predator burden (Gravel and Cooke 2009). This notion is further supported by the apparent correlations between brood abandonment and f_H, and brood size and f_H, which could not be statistically evaluated due to small sample size (Figure 2.5). Future studies are required to further explore the relationship between f_H and reproductive fitness in systems with a greater nest predator burden.

It was further predicted that, since attaining and maintaining dominance requires enhanced energy expenditure, and oxygen required to create ATP is driven by the cardiovascular system (Pörtner and Farrell 2008), f_H would be related to behavioural status in sockeye salmon during competition for dominance on the spawning ground. This prediction was not supported in the present thesis given the lack of apparent change in f_H with shifts in behaviour status, for both males and females. Furthermore, routine f_H did not relate to survival duration during spawning, thus failing to support the notion that elevated cardiac performance would reduce an individual's investment by accelerating the rate of energy expenditure. Previous research has shown that the duration spent at each behaviour status influences energy expenditure and hence metabolic performance (Healey et al. 2003). The relationship between f_H and time spent at each behaviour status was not included in the present study and warrants further exploration.

Overall, neither system (parental smallmouth bass, or competing sockeye salmon) provided direct evidence that overall f_H is related to reproductive behaviours necessary to achieve fitness. In both cases, however, f_H followed a diel pattern, where f_H was 7% and 10% higher during the day than at night, in sockeye salmon and smallmouth bass respectively (Figure 2.8; Figure 3.4). These findings do not follow previously reported behavioural patterns (Hinch and Collins 1991; Cooke et al. 2002a; Healey et el. 2003), but coincide with fluctuations in hormones (e.g. prolactin, melatonin, insulin, gonadal steroids) and blood plasma constituents (glucose, $[Na^+]$, $[Ca^{2+}]$, $[Mg^{2+}]$, $[K^+]$) reported in past literature (McDonald and Milligan 1992; Farrell 1993, Vornanen 2017). Furthermore, for both cases, f_H increased with temperature (Figure 2.2; Figure 3.3). Hence, it is proposed that, instead of being mediated by a single parameter, reproductive behaviour is mediated by the combined effect of f_H and other intrinsic (e.g. blood electrolytes, hormones) and extrinsic (e.g. photoperiod, temperature, and perhaps predation burden as discussed earlier) parameters.

Conclusions

The present thesis reveals the complexity of the relationship between cardiac performance and reproductive behaviours in wild fish. Despite the known enhanced energy demands associated with parental care (Hinch and Collins 1991; Cooke et al. 2002a) and reproductive status during competition for dominance (Healey et al. 2003), and the strong theoretical evidence relating physiological performance to reproductive fitness (Ricklefs and Wikelski 2002; Brownscombe et al. 2017), this thesis failed to demonstrate that an individual's f_H is related to the individual's reproductive behaviours. However, the present thesis demonstrates that combining f_H with other cardiovascular, endocrine or environmental parameters may provide insight on the mechanisms relating physiological and reproductive behaviours. Future studies are required to explore these relationships between behaviour and physiology to further describe the physiological mechanisms underpinning evolutionary ecology and organismal fitness in changing environments.

Future directions

The scope of the present study was restricted to exploring the relationship between f_H and reproductive behaviour. However, other parameters, such as stroke volume and hormones, affect an individual's cardiac performance which could further affect behaviour (reviewed in Gamperl et al. 2017a,b). The large variability in the recorded f_H may have masked trends in the present thesis. Future studies exploring the relationships between physiological performance and fitness should concentrate on investigating trends at the population level and the individual level using different methods, environments, physiological parameters, and combinations of physiological parameters. Suggestions for future directions include:

1. *Continue to explore the relationship between heart rate and reproductive fitness, but begin monitoring earlier in the reproduction phase* – One of the challenges in the present thesis was that the f_H biologgers had to be retrieved to obtain the f_H data. Therefore, to reduce the risk of losing biologgers, fish were only monitored once they had successfully overcome a significant portion of the obstacles preceding spawning (i.e., building a nest and attracting a female smallmouth bass; Ridgway 1988) or migrating long distances with physical barriers (sockeye salmon; Crossin et al. 2004). In consequence, it is likely that the present thesis only included physiologically fit individuals, and neglected the portion of the population with comparatively low routine f_H, potentially causing the relationship between reproductive fitness and f_H to be understated. Monitoring earlier reproductive life history stages could be achieved using f_H telemetry that can transmit the f_H

information to a receiver. However, such devices have yet to be developed.

2. *Explore the effects of compounding stressors on the relationship between physiological and reproductive fitness* – Adding additional stressors, such as light pollution, toxins, noise, and temperature beyond T_{opt}, could accentuate relationships between physiological performance (including f_H). This information could also provide insight on which stressors or combination of stressors are more consequential to a population's fitness, and provide tolerance thresholds. Numerous research has been conducted on investigating the behavioural and physiological responses to such stressors (e.g. reviewed in Iwama 2011), however limited research has investigated the effects on reproductive fitness (e.g. Foster et al. 2016; Maxwell et al. in press), and even fewer studies have explored the compounding effects of stressors (e.g. Whitehead 2013). Quantifying the capacity of a population to cope with different stress scenarios would provide information on the mechanisms driving behavioural responses, that could be further incorporated into evolutionary ecological and bioenergetic models. This could be applied by conservation managers to predict individual and population responses to changing environments, and provide more informed conservation management decisions.

3. *Enhance the resolution of the present studies* – Information on intraspecific cardiovascular performance could be enhanced by incorporating stroke volume or measuring cardiac output. This was not included in the present thesis given that measuring stroke volume and cardiac output in free-swimming fish in their natural habitat is not feasible (methods describe in Steinhausen et al. 2008 and Eliason et al. 2011).

Alternatively, the f_H biologgers could be calibrated to metabolic oxygen consumption (MO_2) to relate metabolic performance to reproductive behaviours, providing a more complete estimate of physiological performance (Eliason et al. 2013). MO_2 data can be easily incorporated into energetic models, which rarely include the metabolic costs of spawning and parental care (Bevelhimer and Breck 2009). Additionally, the behaviour data collected in the current thesis was coarse, resulting in behaviours being pooled into categories for both chapters. Coupling the f_H biologgers with accelerometery data (currently available single unit f_H and accelerometer biologger: DST centi-HRT ACT, StarOddi, Iceland; http://www.star-oddi.com/) would allow f_H to be related to specific reproductive behaviours. The higher resolution could enhance relationships between physiological performance that were otherwise obscure.

4. *Incorporate other physiological parameters when exploring the relationship between heart rate and reproductive behaviour performance* - Given the observed diel pattern in f_H, and the known relationship between the cardiovascular system and the endocrine system, it is suggested that reproductive investment is mediated by f_H in conjunction with other physiological parameters (e.g. energy availability via protein or lipid content, blood [hemoglobin], $[Ca^{2+}]$, blood pressure) (Hendry and Berg 1999; Patterson et al. 2004; Clark et al. 2009). For instance, blood pressure affects the distribution of metabolites to the mussels by influencing membrane ion permeability (Gonzalez and McDonald 1994). Future studies should therefore focus on investigating the relationships between physiological performance and reproductive behaviours at the individual level, using combinations of physiological parameters (e.g. coupling f_H with EMG data and protein

content for metabolic energy reserves (Hendry and Berg 1999; Patterson et al. 2004)).

5. *Determine the minimum required recovery period post-heart rate biologger surgery* – Surgical procedures required to implant f_H biologgers into fish are invasive and stressful for the fish. Multiple studies have used various post-surgery recovery periods, ranging from <12 to 48 hours (e.g. Cooke 2004; Steinhausen et al. 2008; Raby et al. 2015; Prystay et al. 2017). Poor recovery duration can result in elevated routine f_H, skewing natural f_H metrics (Prystay et al. 2017). However, extended recovery periods can add additional stress if the individual is kept in confinement (Portz et al. 2006). As such, there is a need for the development of non-invasive biologgers (in addition to transmitting logger, discussed earlier). In the meantime, however, future studies should investigate the optimal (i.e. minimum) recovery duration for the fish to be fully recovered from the surgical procedure. This would include monitoring f_H recovery to baseline rates, as well as the recovery of other physiological parameters (e.g. cortisol, glucose, and lactic acid; Portz et al. 2006) and behaviour (e.g. swimming performance and reaction times to predators, and prey; Curry and Kynard 1978). Recommended recovery periods should take into consideration fish species, life history stage, size, and environmental factors (e.g. population density, temperature; Wendelaar Bonga 1997; Portz et al. 2006).

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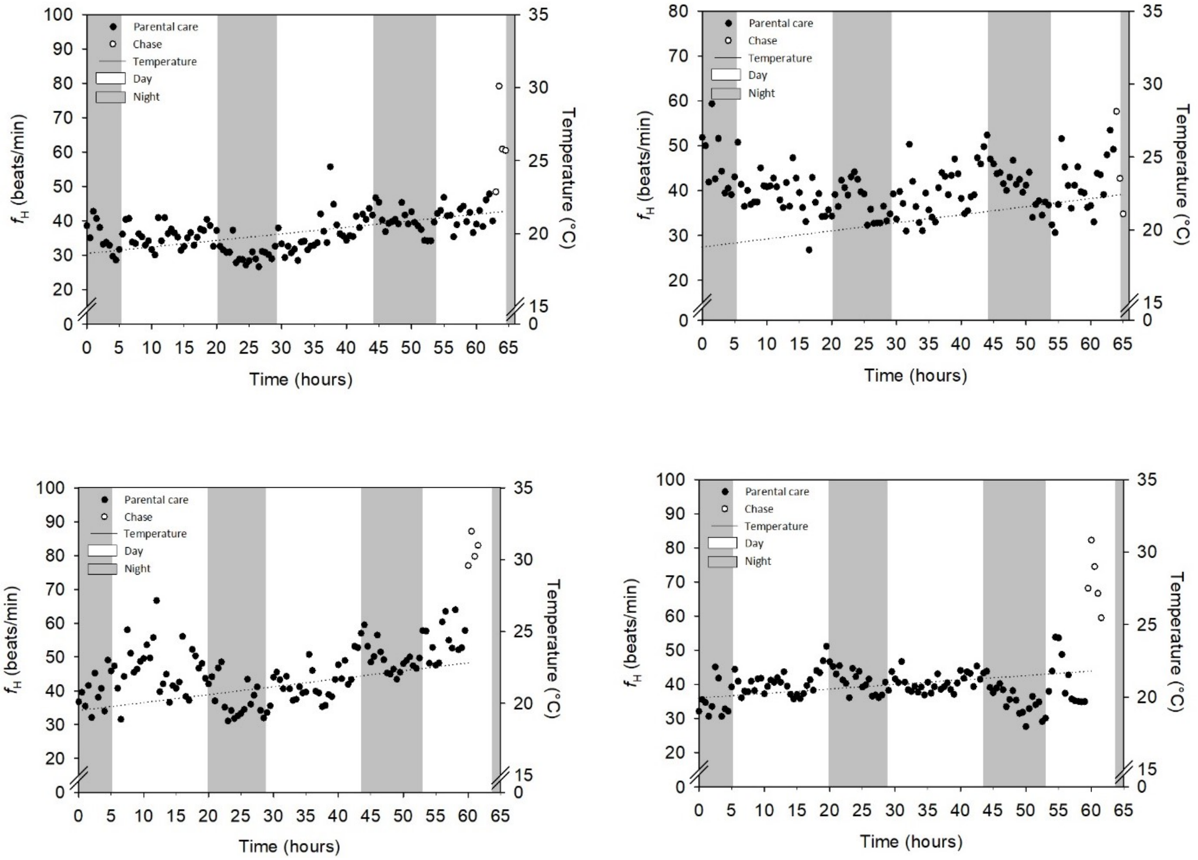
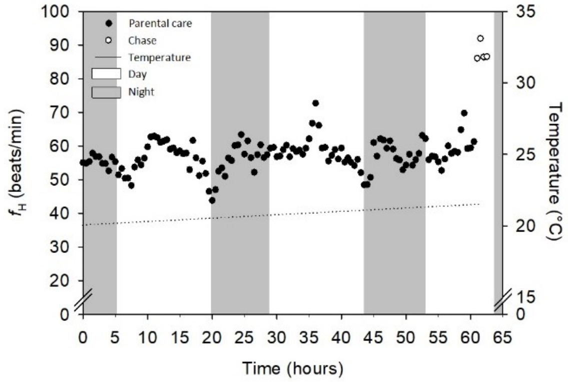
Appendix A. Heart rate (f_H) traces of the 17 smallmouth bass used in Chapter 1. f_H (beats min^{-1}) was averaged for every half hour over a 64 hour period. Black dots represent f_H during parental care. White dots represent f_H during the recapture and chase. Dashed line is the regression line for the water temperature. White bands represent daytime (5:30) and grey bands represent night (20:30).

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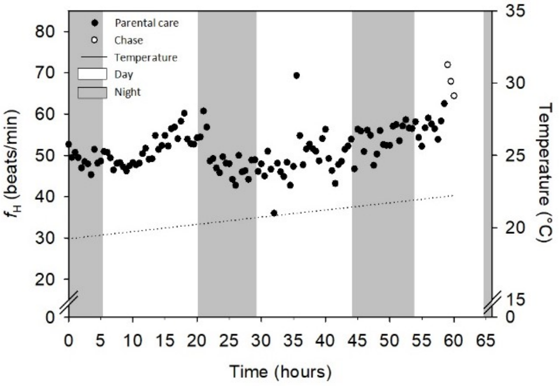
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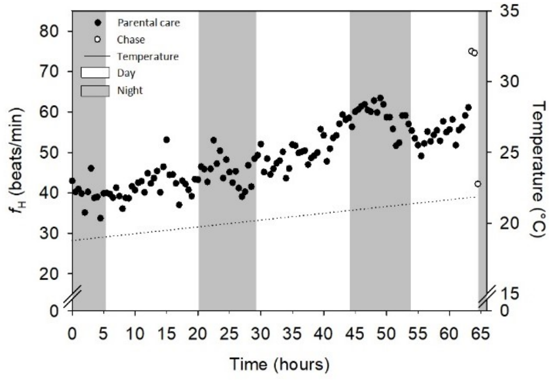
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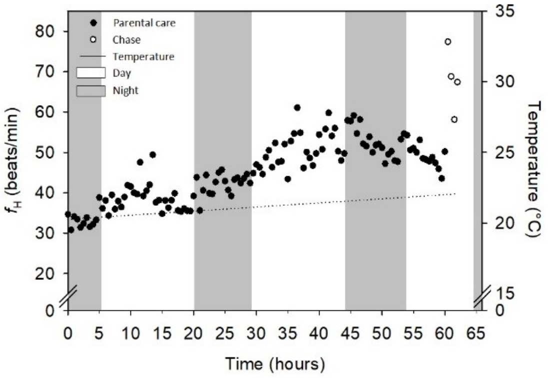
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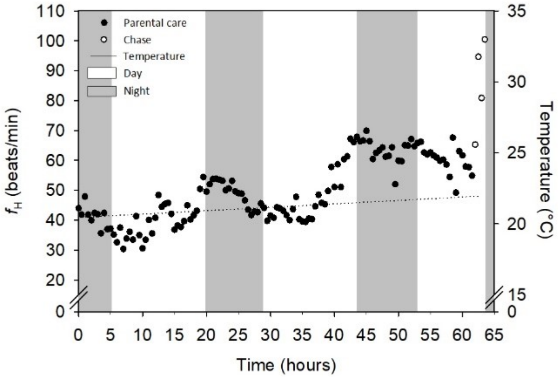
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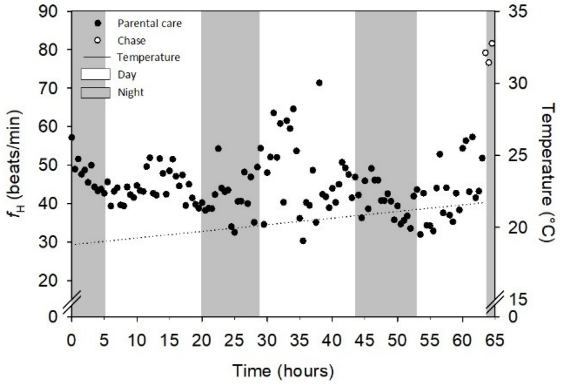
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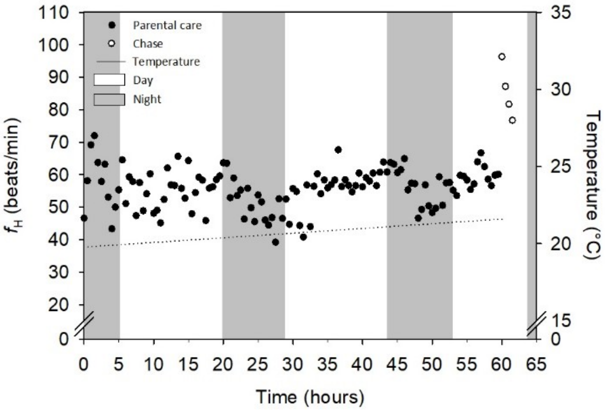
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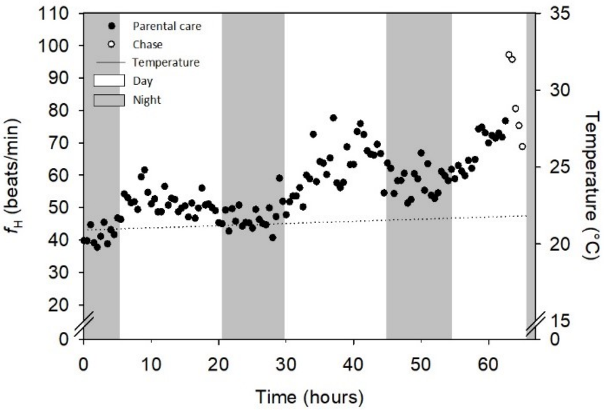
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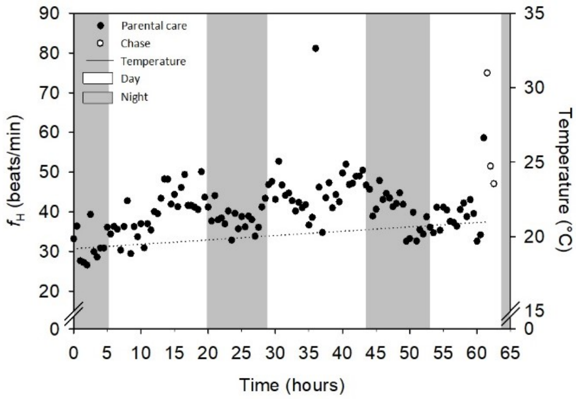
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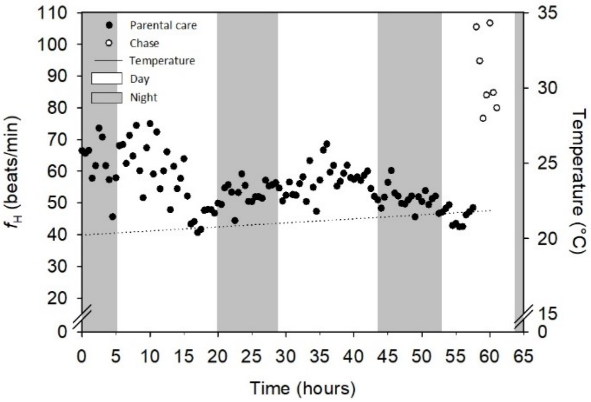
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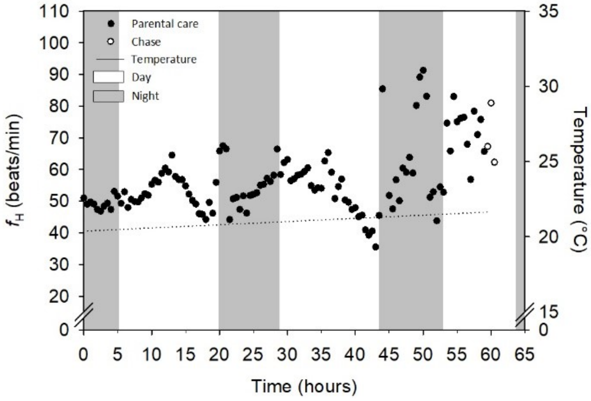
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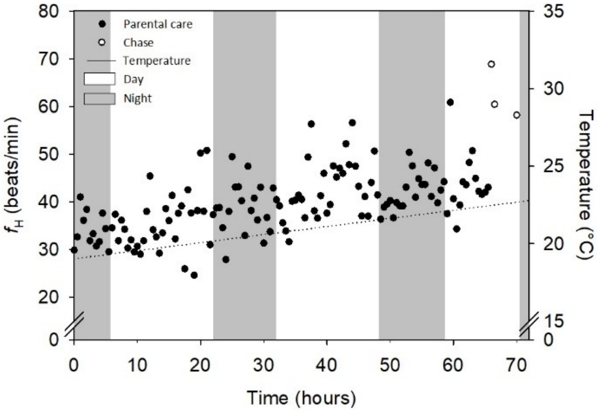
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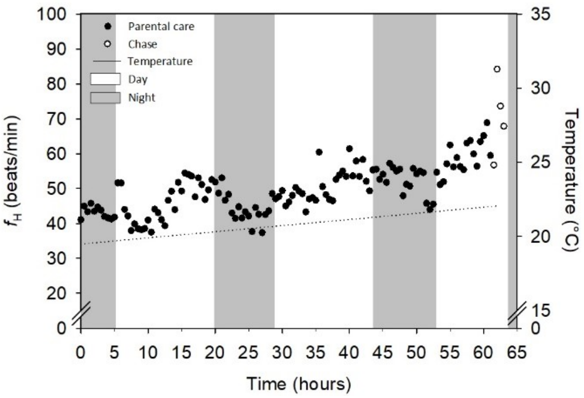

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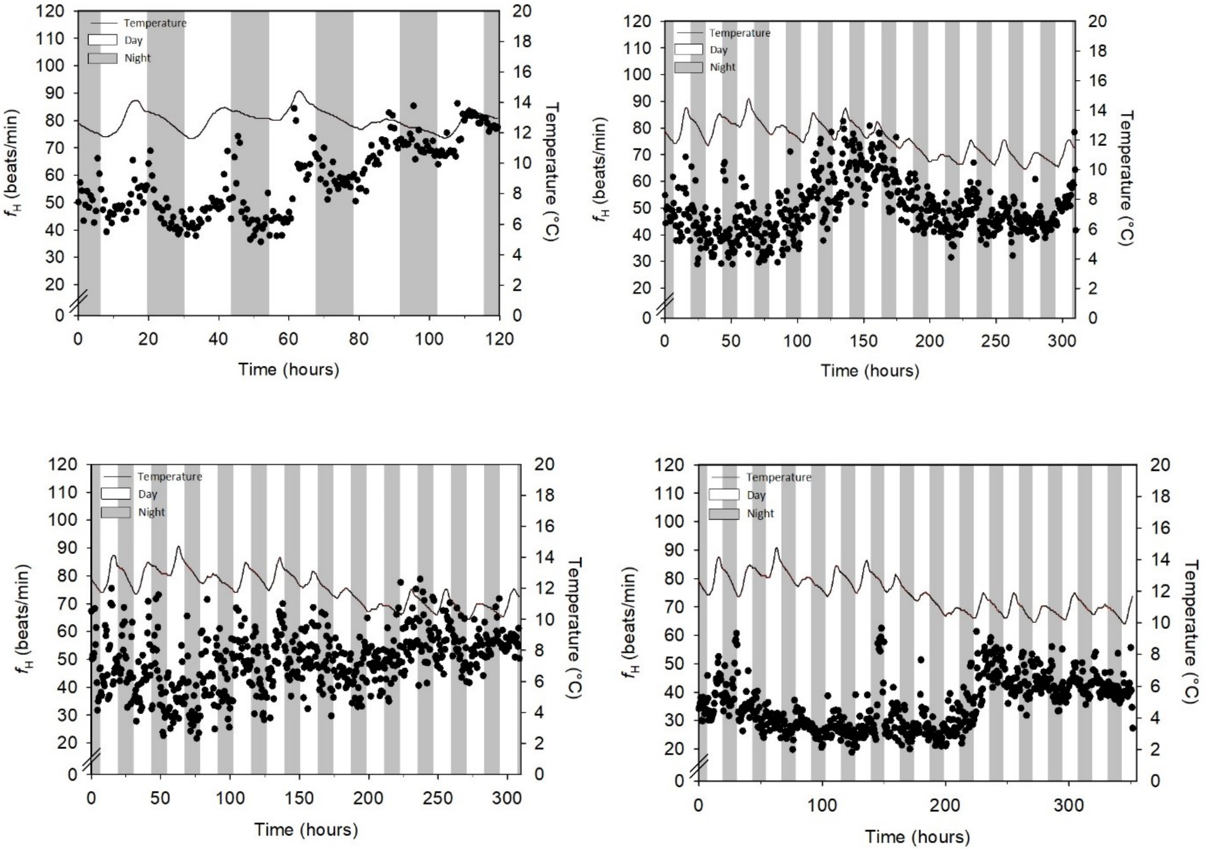
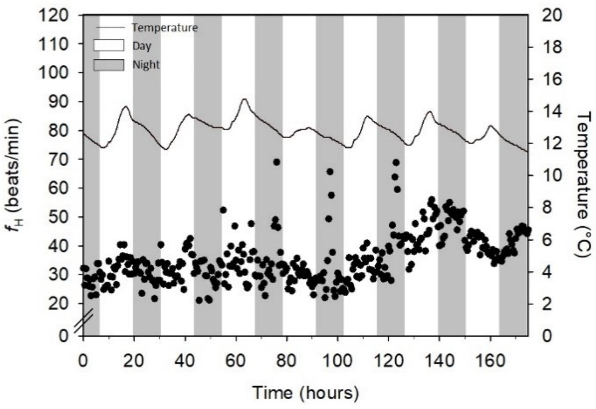
Appendix B. Heart rate (f_{H}) traces of the 55 sockeye salmon used in Chapter 2. f_{H} (beats min^{-1}) was averaged for every half hour. Black dots represent f_{H} during parental care. White dots represent f_{H} during the recapture and chase. Dashed line is the regression line for the water temperature. White bands represent daytime (6:30) and grey bands represent night (20:00).

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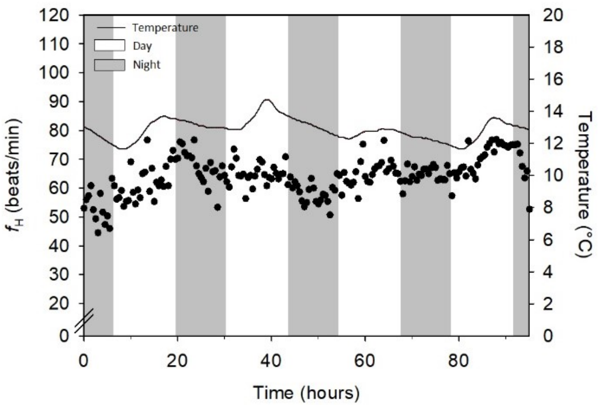
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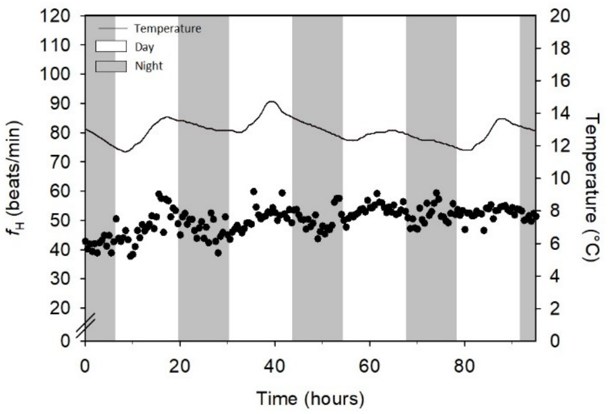
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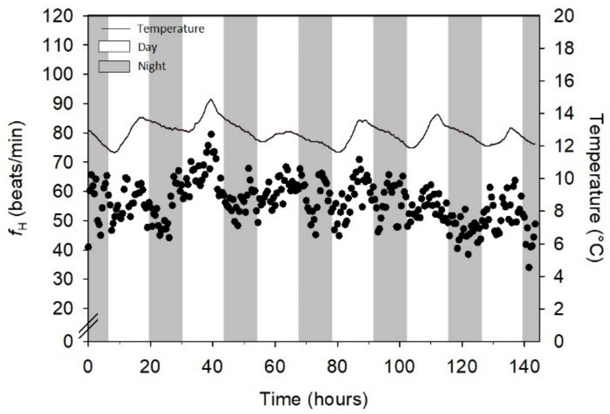
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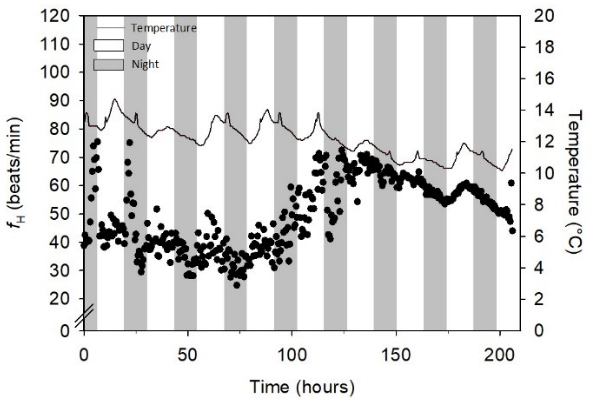
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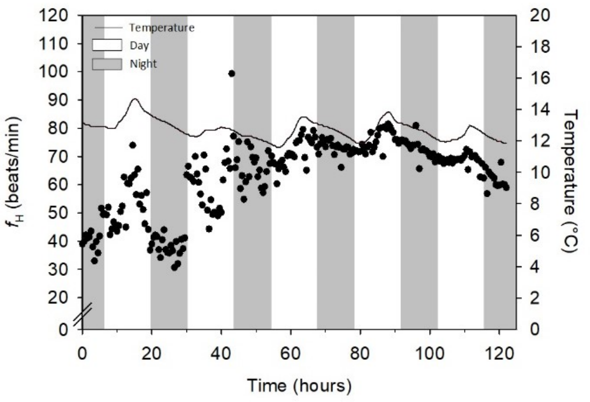
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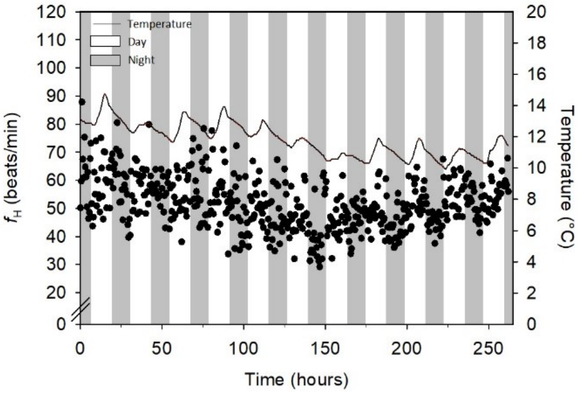
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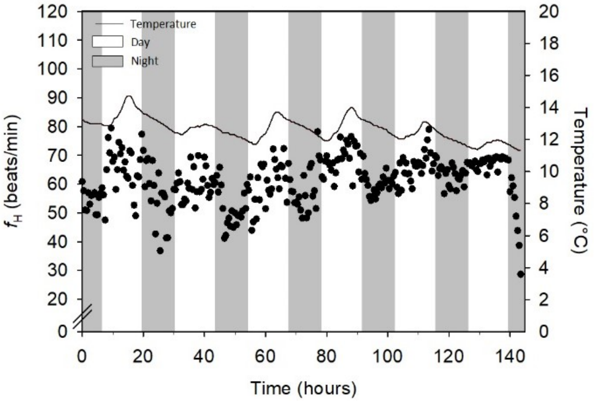
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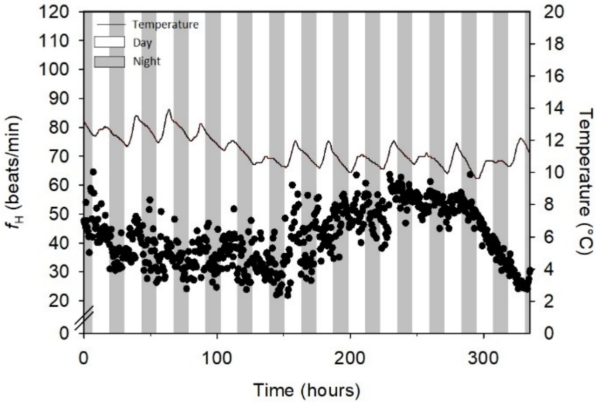
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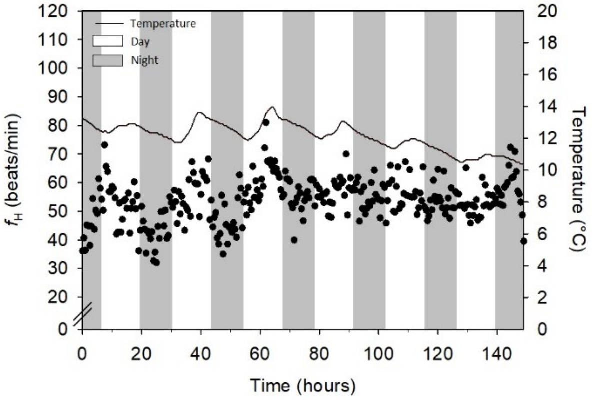
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f_H (beats/min)
Temperature (°C)
Time (hours)
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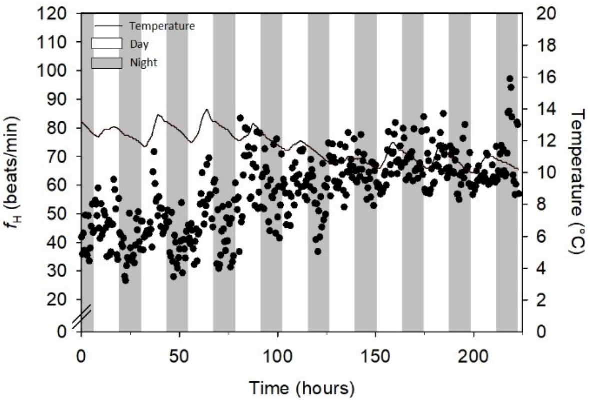
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f_H (beats/min)
Temperature (°C)
Time (hours)
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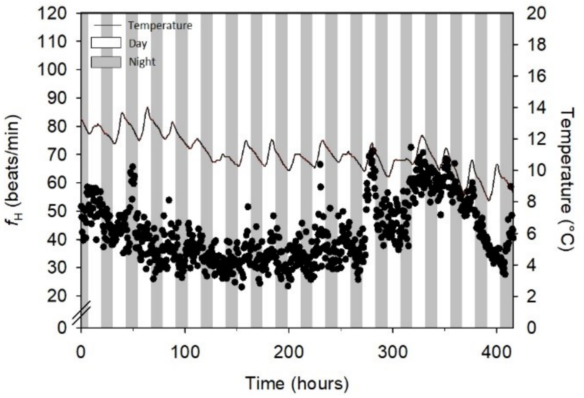
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Temperature (°C)
Time (hours)
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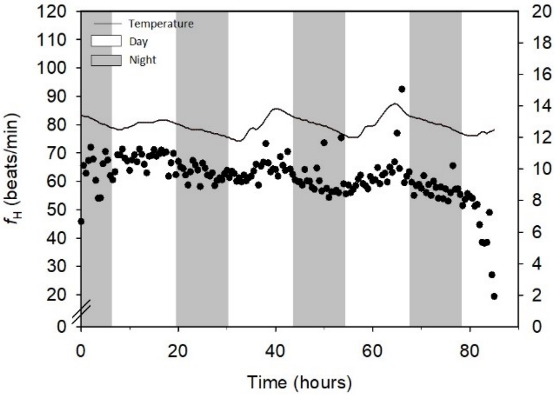
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Temperature (°C)
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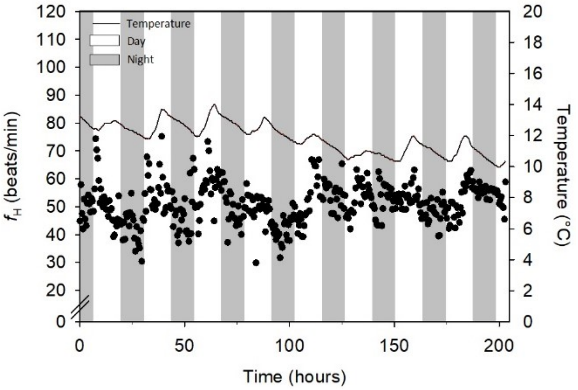
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Temperature (°C)
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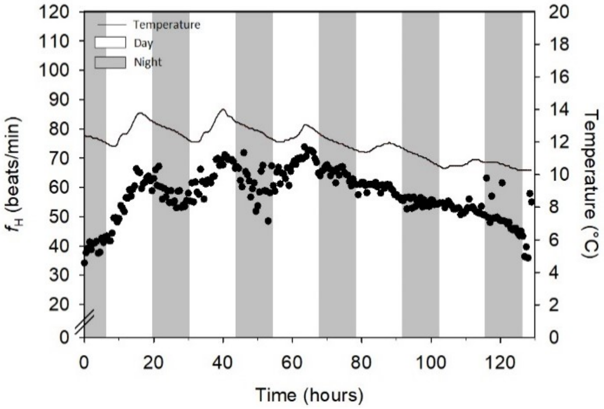
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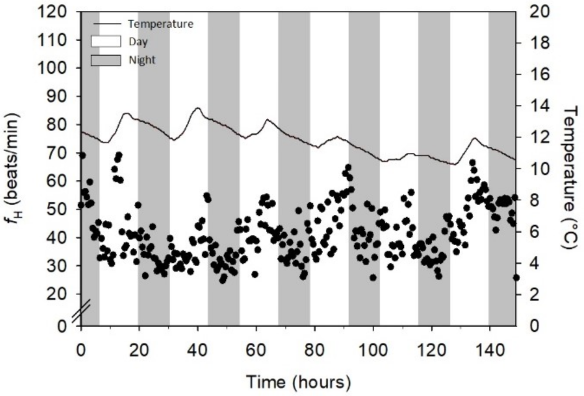
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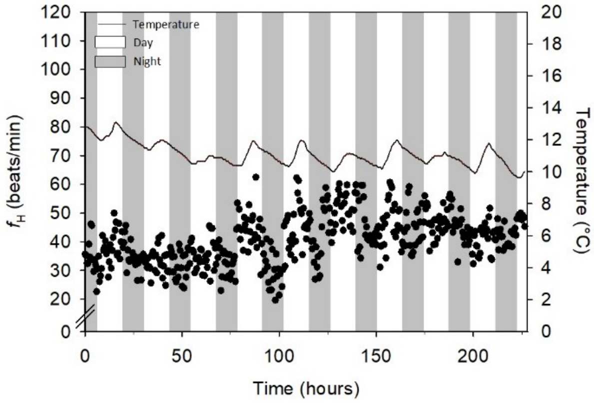
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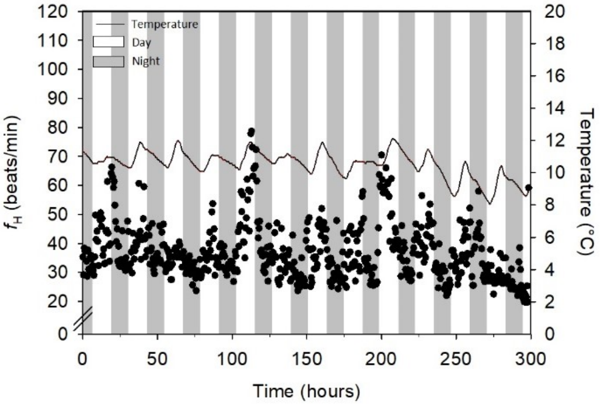
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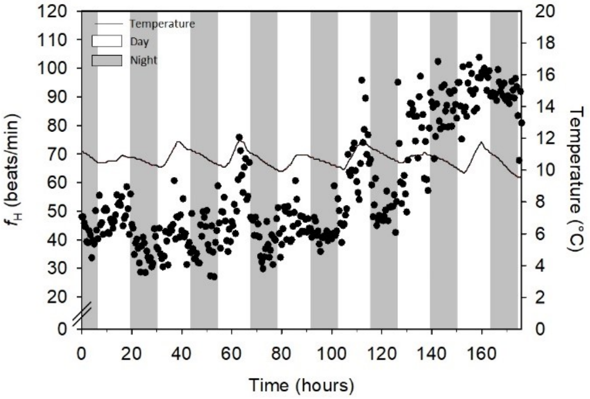
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Temperature (°C)
Time (hours)
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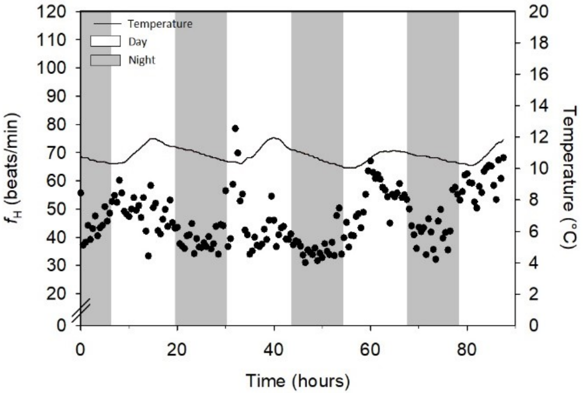
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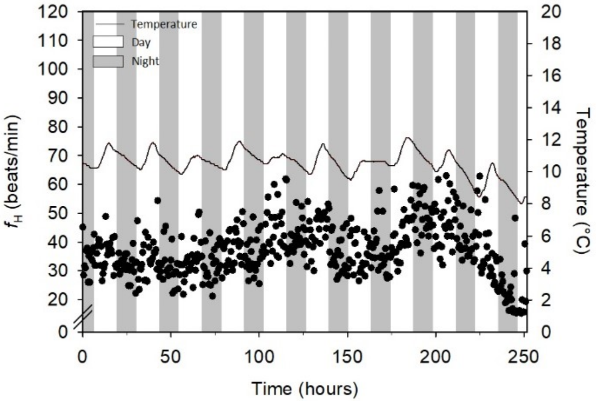
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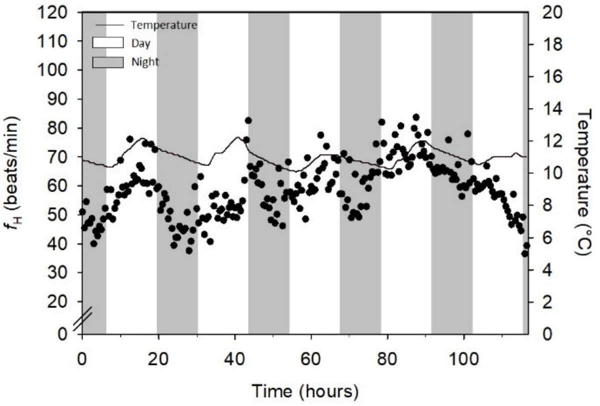
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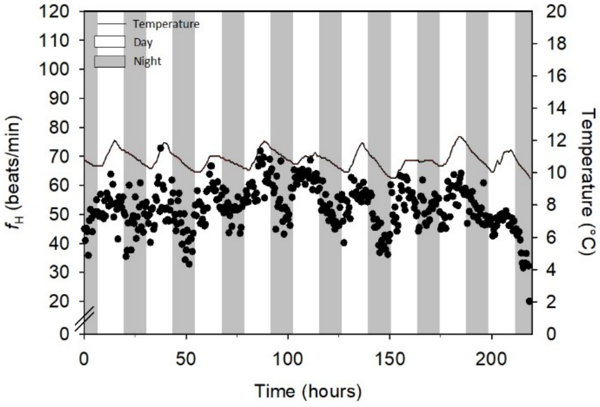
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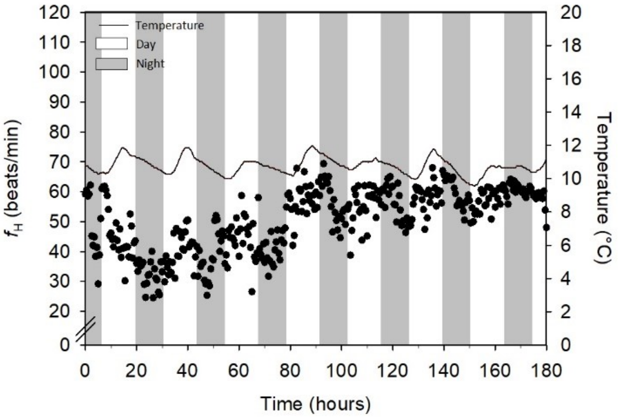
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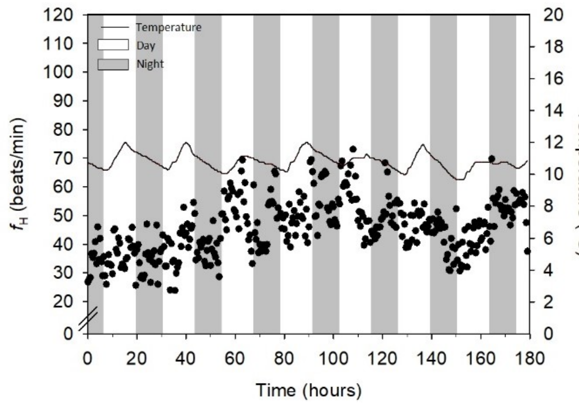
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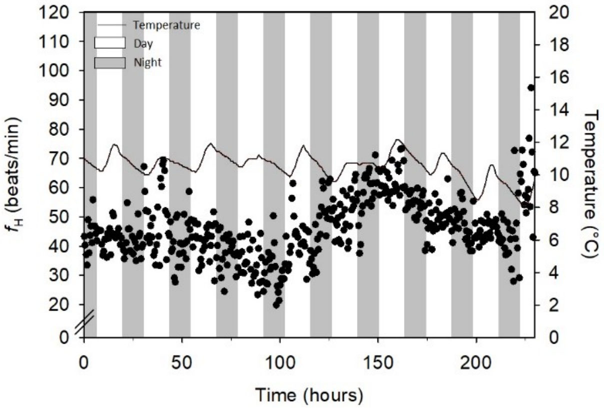
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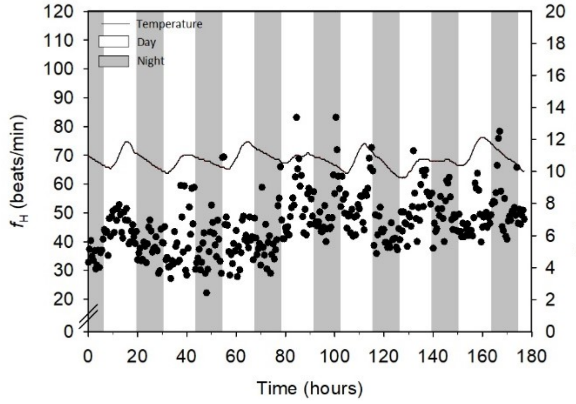
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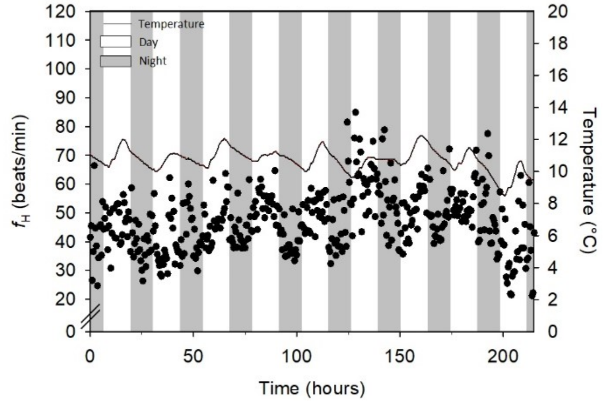
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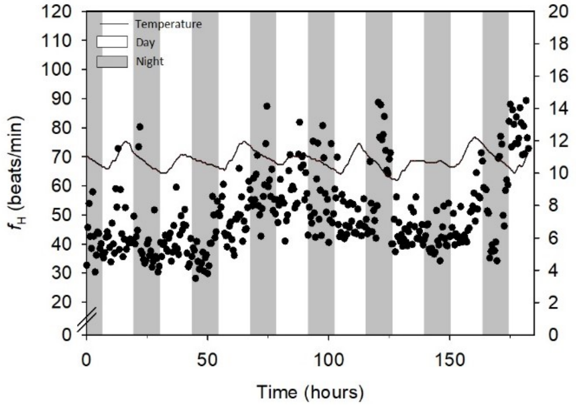
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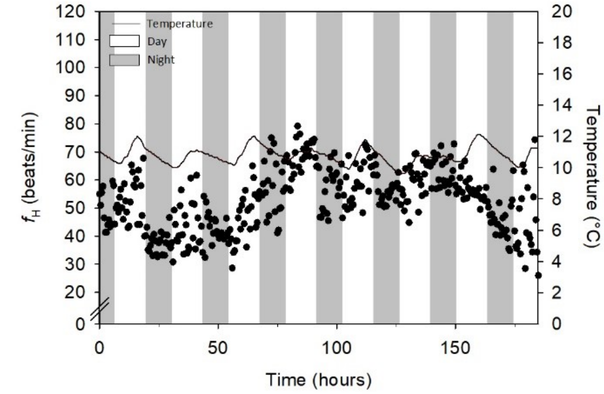
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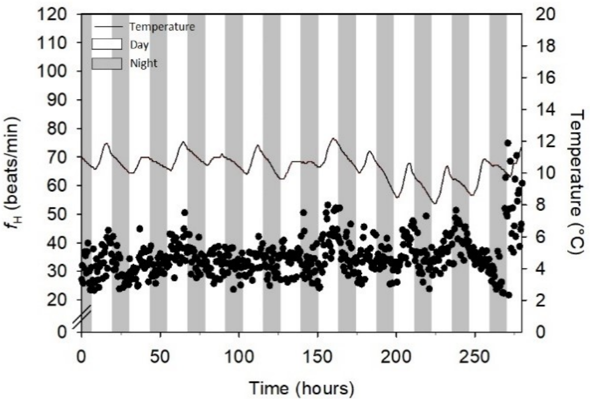

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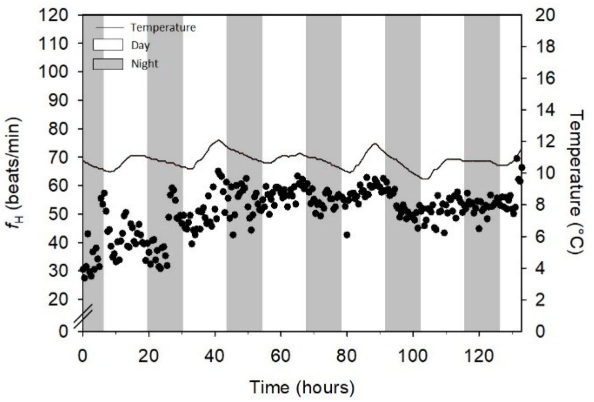

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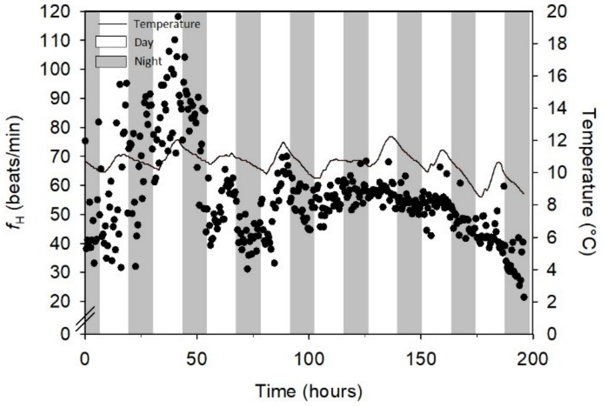

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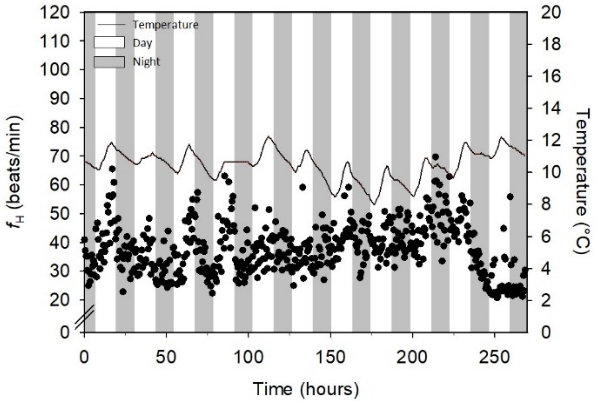

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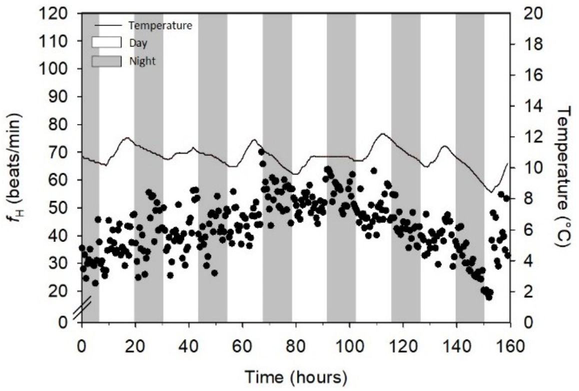

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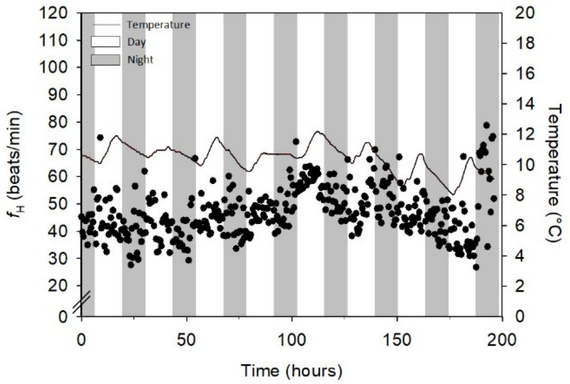

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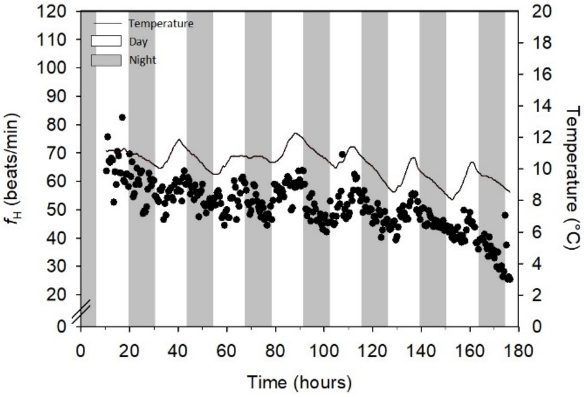
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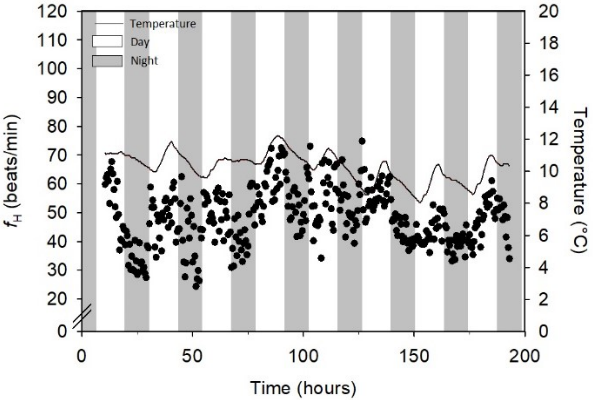
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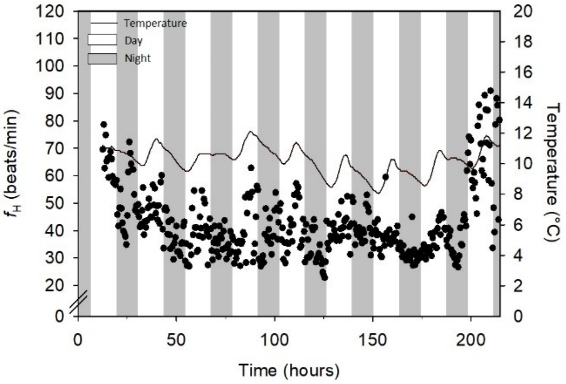
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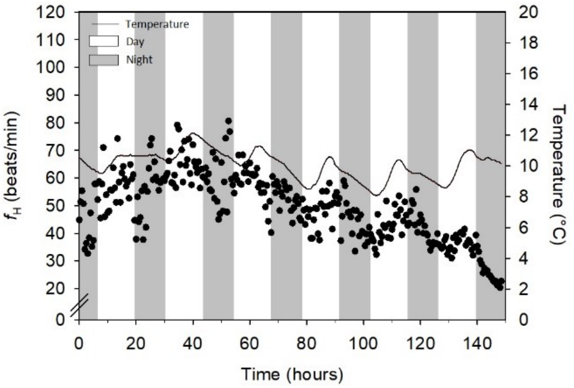
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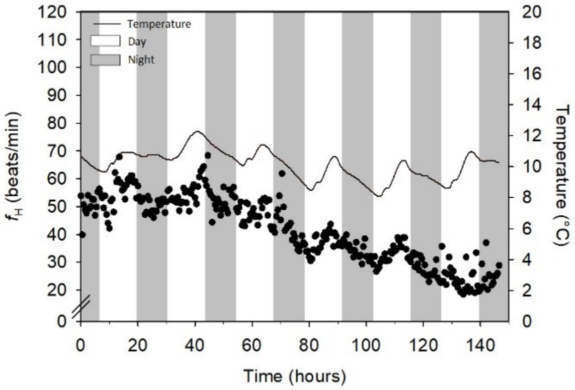
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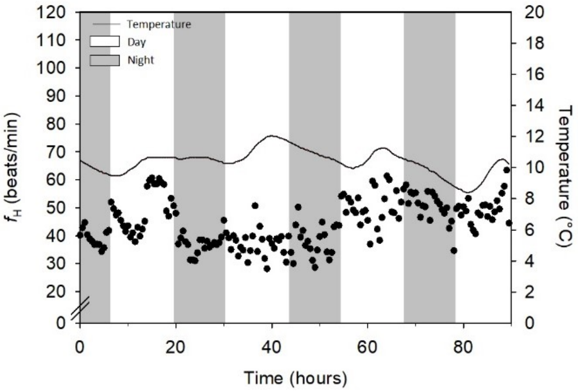
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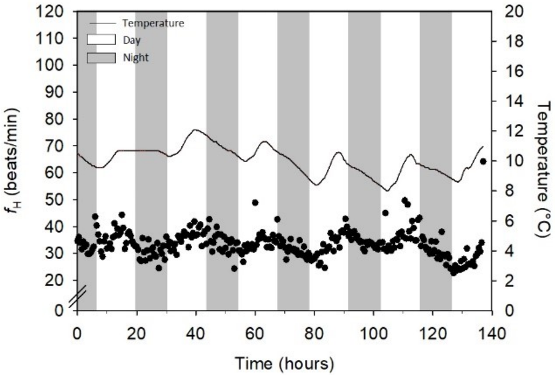
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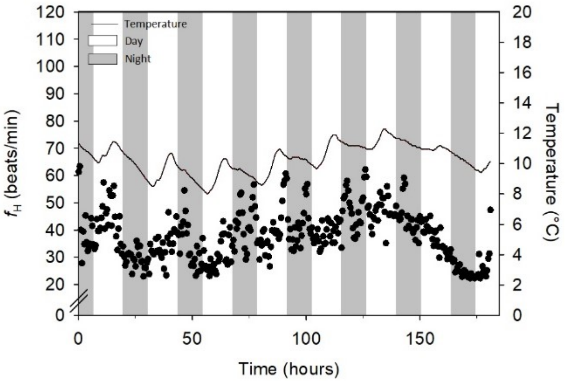
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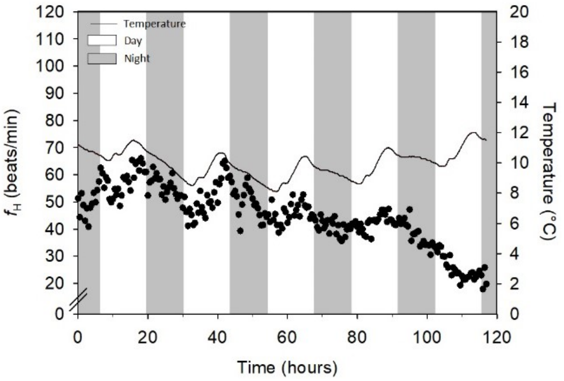
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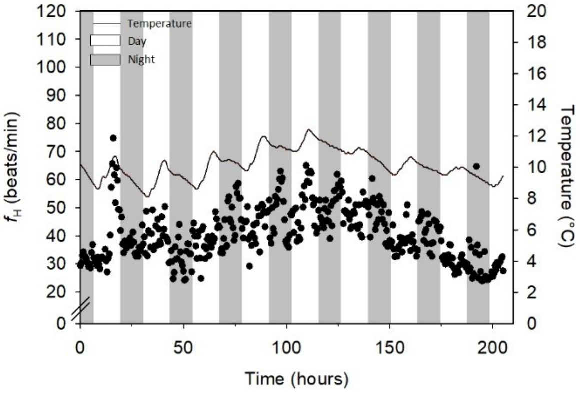
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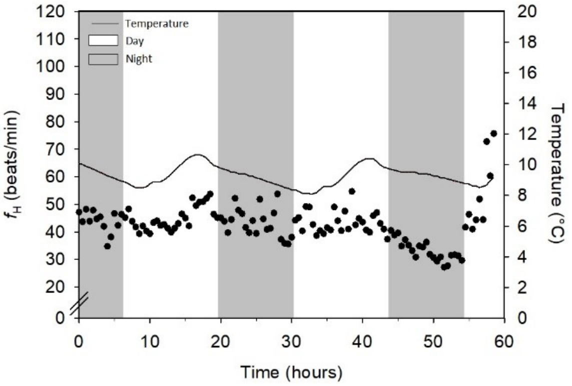
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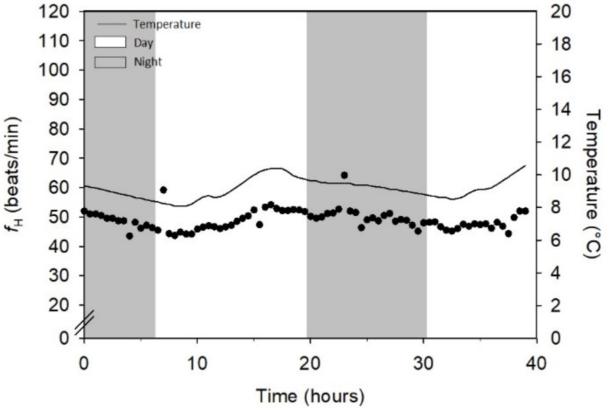
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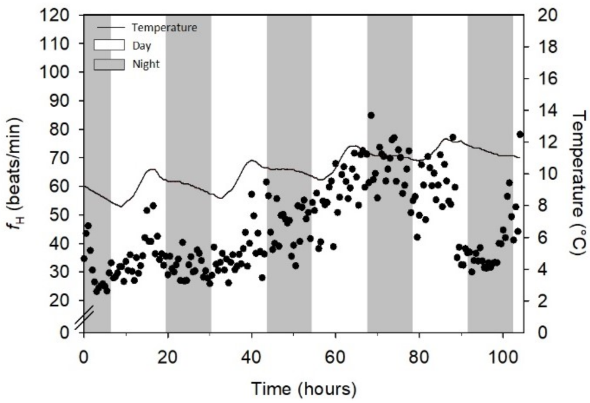
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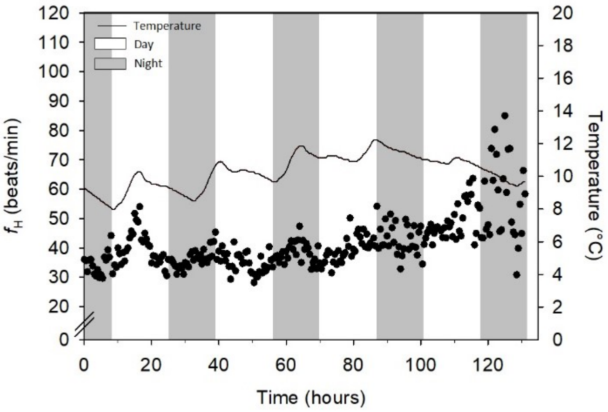
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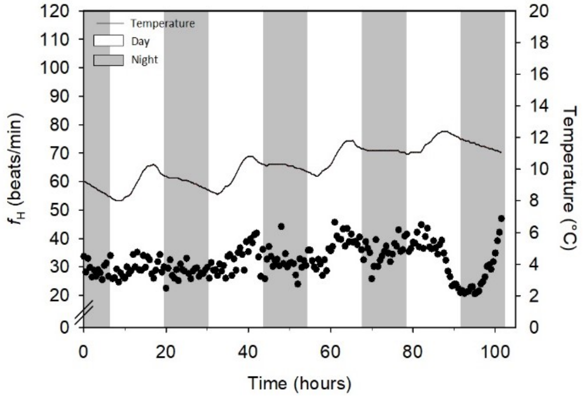
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14
16
18
20
0
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40
60
80
100