

Thesis for the degree of Doctor of Philosophy

EGG SIZE EVOLUTION AND PATERNAL CARE IN PIPEFISHES

Inês Braga Gonçalves

University of Gothenburg
Faculty of Science



Department of Zoology
Animal Ecology
Box 463, SE-405 30 Gothenburg
SWEDEN

The oral defence of this thesis will take place at 10:00 a.m. on Friday September 17th 2010 at the Department of Zoology, Medicinaregatan 18, Göteborg, Sweden.

The opponent is Professor Ian Fleming from Memorial University,
Ocean Sciences Centre, Newfoundland, Canada.

EGG SIZE EVOLUTION AND PATERNAL CARE IN PIPEFISHES

Inês Braga Gonçalves

Department of Zoology, Animal Ecology

University of Gothenburg

Box 463, SE-405 30 Gothenburg

SWEDEN

ines.goncalves@zool.gu.se

Copyright © Inês Braga Gonçalves 2010

ISBN 978-91-628-8154-2

Cover picture composition by Sandi Manhão

Printed by GESON HYLTE TRYCK

Gothenburg, Sweden

From: Baldur ...
Sent: 13 February 2008 17:45
To: 'Inês Gonçalves'
Subject: RE: oxygen

Just for your information, we have identified two ways to make oxygen sensor, where one would be practical for our DST_tags, the oxygen sensor will make the tags very expensive, therefore we have not put development effort into this. This can be done, and we can make such sensors, but you would need to pay for the development cost, where few units would be included. Price for such work would run up to five million Swedish kroner, which I would think is outside your budget?

Development time 2-3 years.

Such work includes:
Development in material technology.
Mechanical construction.
Hardware electronic development.
Software for equipment and PC

I would estimate 3 man years for engineers, plus tooling and other cost.

With best regards
Baldur

Braga Gonçalves, Inês 2010.

EGG SIZE EVOLUTION AND PATERNAL CARE IN PIPEFISHES

SUMMARY

In this thesis I explore how sexual selection, sexual conflicts, coevolution with parental care and an environmental selective agent (hypoxia) affect the evolution of different egg characteristics and embryo survival and size in pipefishes (Syngnathidae).

In the broad-nosed pipefish, *Syngnathus typhle* Linnaeus, both sexes prefer to mate with large partners; large females produce more and larger eggs and large males can care for more young, and, as shown in this thesis, invest more per embryo and provide better oxygenation during brooding. However, if given time to assess their partners prior to mating, females deposit significantly more proteins into eggs when they mate with non-preferred (smaller) males. This result suggests that females are able to improve offspring fitness by compensating for small males' lower paternal care quality, thus reducing effects of differences in care between small and large males.

The spherical shape common to fish eggs minimizes the ratio between surface area and volume. As embryo respiration is assumed to be proportional to egg volume and oxygen availability to be restricted by the surface of the egg, this shape may be unfavourable for larger eggs. In *S. typhle*, both small eggs and hypoxia (low ambient oxygen levels) during brooding resulted in smaller embryos, but the negative effect of hypoxia on embryo size was similar for small and large eggs. In brooding males, within-pouch oxygen levels were found to be consistently lower than those of the surrounding environment, both in high and low ambient oxygen conditions indicating a limited paternal ability to maintain high levels of oxygenation to the embryos.

When comparing pipefish species with and without brood pouches, the species with pouches were found to suffer, on average, greater embryo mortalities during brooding. However, under hypoxia embryo survival and size were negatively affected both in species with and without brood pouches. Between species, large egg size resulted in significantly larger offspring, but had a negative effect on embryo survival, compared to species that produce small eggs.

This thesis contributes to the understanding of egg size evolution in relation to hypoxia and paternal care in syngnathids. The studies presented here suggest that the evolution of brood pouches carries costs in terms of embryo oxygenation. Furthermore, the evolution of more complex types of brooding has potentially enabled phenotypic variation to arise in the quality of male care, in turn selecting for flexible female reproductive investment with stronger selection on egg size evolution in species with brood pouches, and greater female control of egg quality.

Keywords: Egg size, embryo development, embryo survival, hypoxia, male quality, mate choice, mating constraints, oxygen, parental care, parental effects, paternal care, reproductive compensation, sex-role reversal, sexual conflict, Syngnathidae, *Syngnathus typhle*.

LIST OF PAPERS

This thesis is a summary of the following papers:

- Paper I Braga Goncalves, I., Mobley, K. B., Ahnesjö, I., Sagebakken, G., Jones, A. G. & Kvarnemo, C. 2010. Reproductive compensation in broad-nosed pipefish females. *Proceedings of the Royal Society of London Series B-Biological Sciences* **277**: 1581-1587. Doi:10.1098/rspb.2009.2290
- Paper II Braga Goncalves, I., Mobley, K. B., Ahnesjö, I., Sagebakken, G., Jones, A. G. & Kvarnemo, C. Do preferred males provide better parental care in the broad-nosed pipefish? *Manuscript*.
- Paper III Braga Goncalves, I., Ahnesjö, I. and Kvarnemo, C. Effects of egg size and hypoxia on embryo size and survival in a fish with parental care. *Manuscript*.
- Paper IV Braga Goncalves, I., Ahnesjö, I. and Kvarnemo, C. Embryo oxygenation in pipefish brood pouches. *Manuscript*.
- Paper V Braga Goncalves, I., Ahnesjö, I. and Kvarnemo, C. Large pipefish eggs suffer more from hypoxia despite care in male brood pouch: an interspecies comparison. *Manuscript*.

Paper I was reprinted with kind permission from The Royal Society Publishing.

CONTENTS

INTRODUCTION	1
Parental investment and costs of reproduction	1
Parental care, sexual selection and sexual conflicts	2
Parental effects	3
Differential allocation	4
Reproductive compensation	5
The evolution of egg size in fish	6
Why not larger eggs then?	6
Theoretical models of egg size – parental care evolution	6
Parental care and egg size in fish	7
Oxygen availability in water and egg size	7
Effects of hypoxia and parental oxygenation of embryos	9
The family Syngnathidae	11
AIMS	13
METHODOLOGY	14
Experimental fish and breeding setups	14
Oxygen treatments and measurements	15
Behavioural recordings	15
Statistical methods: PERMANOVA+	16
RESULTS AND DISCUSSION	17
Maternal effects and paternal quality	17
Paternal behaviour in relation to oxygen availability, egg size and brood pouch presence	19
Effects of egg size and hypoxia on embryo survival and size	20
Effects of brood pouch and hypoxia on embryo survival and size	21
Effects of brood pouch on egg size variation within and between species	23
CONCLUSIONS	24
Where to go from here?	25
ACKNOWLEDGEMENTS	27
REFERENCES	30
PAPERS I - V	

INTRODUCTION

Most animal life begins in an egg. The reproductive success of an individual is dependent on the quality of the eggs it produces or fertilizes, the success of the development of the embryos inside them, the fitness of the individuals that hatch from those eggs and the likelihood that those individuals will become reproductively mature and will themselves reproduce, either by producing eggs or fertilizing some (or many). Thus, how sexual selection, sexual conflicts, coevolution with parental care and environmental conditions (e.g. hypoxia, temperature etc.) relate to egg quality and offspring fitness, are areas of research that have captured the interest of scientists for decades (e.g. Bagenal 1967; Gross & Sargent 1985; Parker & Begon 1986; Sargent *et al.* 1987; van den Berghe & Gross 1989; Clutton-Brock 1991; Chambers & Leggett 1996; Brooks *et al.* 1997; Hendry *et al.* 2001; Kolm & Ahnesjö 2005).

Syngnathidae (pipefishes and seahorses) is a widespread family composed of mostly marine fishes. Due to their unique modes of parental care and diverse mating systems, pipefishes and seahorses have been extensively studied over the past decades (reviewed in Berglund & Rosenqvist 2003; Wilson *et al.* 2003). The concomitant increase in brooding structure complexity and degree of parental care (Masonjones 2001; Wilson *et al.* 2001), in addition to the large variation in egg size observed between species makes Syngnathidae the ideal model system to study questions concerning the evolution of egg size in relation to parental care and low ambient oxygen conditions. Moreover, the complexity and extent of the care given by brooding fathers to the developing young provide ample opportunities for phenotypic variation to arise in the quality of care provided and this variation may be at the base of female choice and female reproductive allocations in polygamous pipefish species.

Parental investment and costs of reproduction

The evolution of parental care, *i.e.* “any form of parental behaviour that appears likely to increase the fitness of a parent’s offspring” (Clutton-Brock 1991), is an important area in evolutionary biology due to its role in the evolution of mating systems. Differences in the level of parental care provided have contributed to some of the most common differences in mating behaviour between the sexes and to the intensity of competition for mates in both sexes (Trivers 1972; Clutton-Brock 1991).

Life history theory is based on the concept of trade-offs between costs and benefits of reproduction (Stearns 1976). In nature, because resources are limited, individuals are selected to maximise profits for each measure of energy used (Williams 1966), beginning with choosing an adequate partner (Pomiankowski 1987). Reproduction is a highly demanding activity, which not only requires great amounts of resources from both males and females (Sargent *et al.* 1987), it also increases an individual’s risk of injury, predation, parasitism, and of contracting infectious diseases (Clutton-Brock 1991; Magnhagen 1991). In addition, parental care incurs energetic costs to parents, particularly from reduced food intake (De Martini 1987) which in fish often leads to reduced growth during brooding (Svensson 1988; Smith & Wootton 1995;

Jensen 1996; Charnov *et al.* 2001). More recently, focus has been put on understanding direct costs of egg care due to physiological changes that take place in the parents during care (Magee *et al.* 2006; Cooke *et al.* 2010).

Costs of parental care are measured in terms of the reduction in an individual's fitness, that is, the reduction in an individual's reproductive value (Williams 1966; Trivers 1972). Since virtually all forms of parental care incur costs, this consideration gave rise to the concept of parental investment defined by Trivers (1972) as "any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring". This has become a common way of considering the evolutionary consequences of parental care. Reproductive costs can have significant negative effects on the parents' reproductive success on following seasons and thus, can have long term consequences in terms of fecundity or future mating success (Gross & Sargent 1985; Balshine-Earn 1995). Thus, individuals are frequently faced with important reproductive decisions such as: When to reproduce? How much to invest? Who to care for? Parental care evolution theory predicts that a parent should invest more in a brood when its fitness value is greater than any of the alternative investments, such as the parent's own survivorship or future reproductive success (Williams 1966; Gross & Sargent 1985; Sargent *et al.* 1986; Neff 2003). Therefore, if the net benefits decrease through time, strategies such as reduction in parental effort (Manica 2003), nest abandonment (Velez *et al.* 2002) and total or partial filial cannibalism (Lavery & Keenleyside 1990; Manica 2004) are expected by parents in order to minimise their reproductive costs (Smith & Wootton 1995).

Parental care, sexual selection and sexual conflicts

Traditionally, parental care has been considered only to be under natural selection. More recently, however, it has been suggested that sexual selection can select for care and thus influence patterns of parental behaviour (Darwin 1859, 1971; Queller 1997; Kokko & Jennions 2003, 2008). This hypothesis has recently received empirical support in Gonzalez-Voyer and collaborators' study of the evolution of parental care patterns in relation to the intensity of sexual selection in cichlid fishes (Gonzalez-Voyer *et al.* 2008).

No care is thought to be the ancestral state to all other parental care strategies in fishes, that is, male, female and biparental care (Gross & Sargent 1985; Reynolds *et al.* 2002; Mank *et al.* 2005). However, contrary to the scenario observed in mammals where female care is the rule, the transitions between no care and male care in fish are significantly more common than those between no care and the alternatives, suggesting that in different taxa different selection pressures may govern the evolution of parental care in males and females. Lindström & St. Mary (2008) suggested that one such difference may relate to the relative roles natural and sexual selection play in the evolution of male and female care.

Given the costs of egg production (Hinton 1981), a greater share of the female fitness value is dependent on the quality of the male partner (Trivers 1972; Vincent *et al.* 1994), which usually

results in females having lower potential reproductive rates compared to males, hence limiting male potential reproductive rate (Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992). This situation usually causes males to compete more often and energetically for females, whilst females often become the choosier sex, the so called conventional sex roles (Vincent *et al.* 1992). In mammals and to some extent in birds, females provide more care than males, while males compete for mating opportunities with females. However, this pattern is not ubiquitous. As seen in many fish species, the protection and care of eggs within nests do not preclude males from acquiring new mates, that is, paternal care does not necessarily limit male reproductive success (Clutton-Brock & Vincent 1991; Kokko & Jennions 2008; Lindström & St. Mary 2008). Therefore, in many fish systems, males provide all the care despite the fact that they are also the most ornamented sex, and most likely under more intense sexual selection. In fact, fishes are good systems in which to observe examples where female mate choice discriminates in favour of good quality partners in order to maximize fitness returns from female costs of reproduction (Hoelzer 1989; Kokko & Johnstone 2002). For example, in common gobies (*Pomatoschistus microps*) females prefer to mate with males that are already guarding eggs from other females (Reynolds & Jones 1999) and, there is evidence that, in flagfish, pre-spawning fanning functions as a male sexual ornament (Hale & St Mary 2007).

When both sexes invest significantly in a reproductive event, such as in birds with biparental care, or in fish when females produce large eggs and males provide extensive care, as in pipefishes and seahorses, conflicts may arise as to how much each sex should invest or as to how each sex should respond to changes in investment by the other sex (Parker 1979; Royle *et al.* 2002; Lessells 2006). Conflicts between the sexes are important driving forces in the evolution of both mating systems and reproductive behaviour, and therefore can have important consequences for offspring fitness (Alonzo 2010). Sexual conflicts may result from postcopulatory sexual selection, in which the sex enforcing it controls the fitness payoffs of a particular breeding event at the cost of its mate's fitness (Paczolt & Jones 2010). Between breeding events, postcopulatory sexual selection and other parental effects can mediate trade-offs between investment in current and future reproduction, allowing individuals to maximize their reproductive fitness over time (Marshall & Uller 2007; Paczolt & Jones 2010).

Parental effects

Parental effect is the term given to the causal influence of the genotype or phenotype of the parent on the offspring phenotype (Wolf & Wade 2009). Such effects can be divided into paternal and maternal, although the latter are the most commonly studied. Parental effects may have genetic or environmental sources, but both kinds of effects affect the offspring through its environment. Genetically-based parenting ability has the potential to directly affect an offspring's phenotype, regardless of it being inherited. Also, because the fitness value of a brood may be dependent on the environmental conditions and their impact on parental investment, parental effects may result from indirect environmental effects (Marshall & Uller 2007). For

example, poor environmental conditions may require a higher investment from the parent to provide the same amount of care compared to more benign conditions. Thus, through parental effects, individuals can match the phenotype of their offspring to the environmental conditions they experience (Mousseau & Dingle 1991; McCormick 2003), either buffering offspring from poor environmental conditions (Agrawal *et al.* 1999) or lowering offspring fitness in response to deterioration of the environment (Bernardo 1996a). In contrast, when conditions are favourable, parents are predicted to increase their reproductive effort, because the net benefits are higher when there are readily available resources (Pilz *et al.* 2003). In these situations, females may be expected to produce heavier or larger clutches and males to care for longer periods of time, or to increase the care given at any time to its offspring, thus increasing the embryos' chances of surviving until hatching.

Individuals are also able to influence their offspring phenotypes in response to the perceived quality of their partners and to the expected fitness benefits from a particular breeding attempt (Burley 1988; Davies & Hatchwell 1992; Sheldon 2000; Gowaty 2008).

Differential allocation

In iteroparous species (*i.e.* which reproduce more than once) individuals are expected to reproduce only when the advantages of breeding at that particular time, outweigh the costs of reproduction in general (Neff 2003). Thus, when encountering mates of poor quality, reproduction should be deferred until a better quality partner can be found or until the subsequent breeding season. In contrast, when the mate encountered is above average quality, an individual should invest more in that particular reproductive event, even at the cost of reduced future survival and lower future reproductive value (Williams 1966).

The Differential Allocation Hypothesis (DAH) was first described by Burley in 1986, as behaviours that individuals undertake in order to increase their chances of acquiring and maintaining good mates. This hypothesis is based on the following assumptions, applicable to both sexes:

- Reproductive investment must be influenced by mate attractiveness so that reproducing with such a mate must be worth the costs of reproducing at all;
- Parents must trade current and future reproductive attempts, *i.e.*, increased allocation in current reproduction must incur a cost in terms of future reproduction.

According to a broader definition of this hypothesis (Sheldon 2000), attractive partners are those who provide better or more suitable habitat or care, or those who produce more or better eggs. In addition, attractive mates may pass on "good" genes to their offspring increasing their fitness, either through greater offspring viability (Trivers 1972; Zahavi 1975) or greater offspring attractiveness (Fisher 1930; Lande 1981). In iteroparous species, individuals that are able to regulate their reproductive investment in accordance with their mate's attractiveness

(Loyau *et al.* 2007) should be at an advantage in relation to individuals which allocate resources to reproduction irrespective of mate quality (Sheldon 2000).

Reproductive compensation

Naturally, individuals may suffer from ecological or social constraints that reduce their ability to find and acquire good quality mates or of surviving until the subsequent breeding season (Gowaty *et al.* 2007; Gowaty 2008). Demographic stochasticity, limitations to dispersal and risk of predation are important ecological factors that may restrict an individual's ability to freely choose a mating partner from the population (Gowaty 2008). Male-male competition and male coercion, among others, are social factors that can specifically hamper a female's capacity to mate with a preferred partner (Gowaty *et al.* 2007). Unable to find suitable partners, individuals may be constrained to mate with less preferred partners, which often results in less viable offspring (Sandvik *et al.* 2000; Bluhm & Gowaty 2004a; Anderson *et al.* 2007). To minimise this cost, individuals may reproductively compensate for low offspring fitness either by increasing their fecundity (Gowaty *et al.* 2007), improving the nutritional content of their gametes (Bluhm & Gowaty 2004b), or by improving or extending the length of parental care provided to those offspring (Byers & Waits 2006).

In general, reproductive compensation should even out parental productivity (number of offspring reaching reproduction) of constrained individuals to that of unconstrained individuals, which are able to mate with preferred partners (Gowaty *et al.* 2007; Gowaty 2008). Reproductive compensation should occur in systems where:

- Mate preferences translate into variation in viability and fitness of the resulting offspring;
- Social and/or ecological constraints limit opportunities to mate with preferred partners;
- Differences in the ability to compensate reflect environmental and developmental factors, which may affect individuals differently (Gowaty 2008).

Compensation, however, whether through increased fecundity or through any other mechanisms, is a costly activity, thereby reducing the fitness of compensating individuals (Moore *et al.* 2003).

Despite enjoying empirical support, both the differential allocation and the reproductive compensation hypotheses are under debate (Ratikainen & Kokko 2010). The first theoretical treatment of these hypotheses was recently published (Harris & Uller 2009). It showed that both positive and negative reproductive allocations can arise from the same principles, which strategy is adopted may depend on small adjustments of parameters such as the effect of parental effort on offspring survival (Harris & Uller 2009). However, this is currently a vivid area of research and, given the increased interest in this type of parental effects, further empirical and theoretical contributions should be expected in the near future.

The evolution of egg size in fish

Egg size can vary immensely among species, populations within species, females within populations, and also within and between breeding seasons (Ernsting & Isaaks 2000; Fox & Czesak 2000). Initial egg size is a crucial factor determining survival in many organisms (Kaplan & King 1997). Early size advantages can be additive and may be amplified throughout development (Vigliola & Meekan 2002), with larger egg sizes giving rise to larger larvae or juveniles (Duarte & Alcaraz 1989) and conferring them an advantage compared to smaller larvae and other life-stages (Roff 1992; McCormick & Hoey 2004). Specifically in fish, larger egg sizes have been linked to larger sizes at emergence (Ahnesjö 1992a), greater early survival (Henrich 1988), faster growth (Fowler 1972), lower predation rate (Bilton *et al.* 1982; Ahnesjö 1992b) and greater adult sizes with consequent greater reproductive output (Holtby & Healey 1986; van den Berghe & Gross 1989).

Why not larger eggs then?

Apart from rare exceptions (e.g. dogfish), eggs of aquatic organisms do not become as large as those of birds or reptiles. If the production of larger eggs gives rise to so many benefits, why don't we observe generally larger egg sizes among aquatic organisms, and specifically among fish, than we do? The production of large eggs can incur significant costs on the parents. Firstly, the energetic costs of producing eggs are generally great and thus, larger eggs are nutritionally more expensive for females to produce (Wootton 1990), leading to a trade-off between size and number of eggs that can be produced with the same reproductive effort (Duarte & Alcaraz 1989) or, if numbers are kept constant while size increases, a trade-off between current and future reproduction. In addition, larger eggs tend to have longer developmental times, thus requiring longer incubation periods, and potentially, parental care (Balon 1984). A second trade-off may occur if mortality during the egg stage increases with egg size. This may occur if instantaneous offspring mortality is constant through time and offspring from larger eggs spend proportionally longer time at the egg stage, compared to offspring from smaller eggs or, if larger eggs suffer higher instantaneous mortality rates compared to smaller eggs, due to limitations in acquiring available oxygen (Sargent *et al.* 1987).

Theoretical models of egg size – parental care coevolution

The relationship between parental care and egg size in aquatic organisms has always been contentious and important to our understanding of reproductive strategies (Clutton-Brock 1991; Stearns 1992), having inspired a multitude of theoretical models on life history evolution.

Shine (1978) developed a model based on his “safe-harbour” hypothesis. In a scenario where the embryonic stage has lower mortality than the juvenile stage due to parental care, natural selection should favour an increase in the time spent as an embryo and a decrease of the juvenile stage. This could be achieved by increasing egg size so that most of the development into adulthood would occur at the egg stage (Shine 1978).

In contrast, Nussbaum (1985, 1987), proposed a model in which egg size precedes the evolution of parental care, potentially through selection for specific juvenile traits such as the ability to consume large food items, increased competitive ability (Nussbaum 1981) or greater predation avoidance (Kerfoot 1974; Ahnesjö 1992b). Such selection would result in higher embryo mortality due to the longer development times, and to the production of fewer eggs due to energetic restrictions, resulting in higher embryonic and relative juvenile mortality. Hence, behaviours that would increase egg survival, offsetting the costs of larger eggs, should be selected, that is, parental care should be selected and favoured. Despite strong criticism (Shine 1989), this possibility received some support in a study by Summers *et al.* (2006), who concluded that, in frogs, the evolution of large egg sizes have typically preceded the evolution of parental care.

Nussbaum & Schultz (1989) later proposed that once both factors were present, parental care and an increase in egg size, these should begin to co-evolve so that it would be difficult to disentangle the selective forces driving the evolution of each factor. According to this model there should be no clear trends in the order of parental care and egg size evolution.

Parental care and egg size in fish

Parental care has been repeatedly associated with larger egg sizes in fishes, both between (Shine 1978; Gross & Sargent 1985; Sargent *et al.* 1987; Kolm & Ahnesjö 2005) and within species (van den Berghe & Gross 1989). For example, species that provide parental care tend to produce larger eggs than broadcast spawning species (Goodwin *et al.* 2002). Furthermore, a positive correlation between quality of parental care and egg size has been reported in several fish families (Gross & Sargent 1985). Parental care has, however, also been linked to factors other than egg size (Kolm *et al.* 2006). Kolm *et al.* (2006) provided the first phylogenetically-based comparative analysis that focused on the links between egg size, clutch size, parental care and body size in cichlids. Their results showed that clutch size had the largest effect on care duration whilst no positive link could be found between egg size and duration of care. Nevertheless, parental care seems to be the single most important factor influencing egg size and affecting embryo survival in marine organisms (Coleman 1992). Parental care improves offspring development (Sargent 1997), can affect offspring phenotype and enhances survival (Sabat 1994).

Oxygen availability in water and egg size

Aquatic environments differ from terrestrial ones in multiple ways, including lower and more variable levels of dissolved oxygen (Keister *et al.* 2000). In natural waters, oxygen transport occurs through two main ways: diffusion and convection. While convection occurs when oxygen is carried along with the water and therefore can travel long distances, diffusion is the movement of individual molecules. Above a few millimetres, diffusion becomes too slow a process because the oxygen diffusion coefficient in water is approximately one million times lower than in air

(reviewed in Rombough & Randall 1988). As a result, localised declines in oxygen availability, particularly in sheltered shallow bays commonly take place, potentially causing hypoxia, that is, any level of dissolved oxygen that negatively affects the physiology or behaviour of organisms (Pollock *et al.* 2007) or anoxia (0 mg O₂/litre). At low dissolved oxygen levels, which under natural conditions can occur in the early morning as a consequence of plants and algae ceasing photosynthesis overnight, while respiration still takes place, embryo mortality of demersal eggs can be high from suffocation (Alderdice *et al.* 1958; Kamler 1992). Embryos from demersal eggs are particularly sensitive to hypoxia because they lack the ability to move away from poorly oxygenated areas (Pelster 1999). Thus, ambient oxygen levels may be an important selective agent in aquatic environments and suitable to manipulate experimentally to better understand egg size and parental care adaptations.

Fish eggs are composed of several layers (Rombough & Randall 1988). The egg shell prevents the embryo from being in full contact with the surrounding environment during development, but also restrains oxygen transfer between the embryo and the external environment. In addition, the shell creates two other barriers that further restrict the passage of oxygen to the embryo: the external boundary layer and the perivitelline fluid. The boundary layer is a semi-stagnant layer of water that surrounds the egg, where oxygen is quickly depleted, and metabolic wastes from the embryo accumulate. The water velocity necessary to stir the boundary layer is directly proportional to the rate of oxygen consumption and therefore increases steadily throughout development (Daykin 1965). Smaller eggs generally have lower metabolic rates and thinner boundary layers (Wickett 1954). The perivitelline fluid fills the space between the inside of the egg shell and the embryos, and constitutes the biggest barrier to oxygen diffusion inside the eggs (Berezovsky *et al.* 1979).

Fish eggs are typically spherical, which means that they have an unfavourable surface area to volume ratio, with increases in egg size resulting in greater increases in volume compared to the surface area. Oxygen demands of embryos are mostly assumed to be proportional to egg volume (but see Einum *et al.* 2002; Rombough 2007). However, because oxygen availability is restricted by the surface area of the egg (Hendry & Day 2003; Kolm & Ahnesjö 2005), embryos from larger eggs are assumed to have greater difficulties acquiring oxygen for successful development compared to embryos from smaller eggs (van den Berghe & Gross 1989; Quinn *et al.* 1995). These considerations have led to the general assumption that large embryos require more oxygen than they can acquire by diffusion and cannot develop, hence causing a restriction on the evolution of egg size in aquatic environments. This assumption has been extended to predict that embryos from larger eggs suffer from higher mortality in low oxygen conditions (Kolm & Ahnesjö 2005).

Despite widely accepted, this assumption has had mixed support. Egg clutches of marine invertebrates (Crisp 1959; Naylor *et al.* 1999; Fernández *et al.* 2002) are oxygen limited and in marine fish, peripheral embryos develop faster than central ones in three-dimensional egg clutches (Coleman 1992) because oxygen diffusion becomes restricted in the centre. Furthermore, in the mouth brooding cichlid, *Pseudocrenilabrus multicolour victoriae*, females

from populations that naturally experience hypoxic conditions produce smaller but more numerous eggs than females from populations that experience stable high oxygen conditions. Surprisingly, however, F1 females both from high ambient oxygen and from hypoxic populations produce larger eggs in hypoxia than in high ambient oxygen conditions (Reardon & Chapman 2009). Moreover, while small *Amphiprion melanopus* eggs require 33% less oxygen during development compared to larger eggs from the same clutches (Green *et al.* 2006), brown trout embryos from small eggs were found to have lower survival in low oxygen conditions compared to embryos from larger eggs (Einum *et al.* 2002), generating some controversy as to which egg size is more negatively affected by hypoxia.

Effects of hypoxia and parental oxygenation of embryos

Developing embryos are extremely sensitive to hypoxia (Doudoroff & Shumway 1970) and such sensitivity tends to increase throughout development (Rombough 1988) due to increasing oxygen consumption (Green 2004; Green & McCormick 2005). Particular responses of embryos to hypoxia depend on species, stage of development, level of hypoxia, and duration of exposure. The amount of dissolved oxygen in the water can affect the metabolism and development of fish embryos (Rombough & Randall 1988; Pelster 1999; Reardon & Chapman 2009) and consequently their survival. Hypoxia can reduce metabolism (Bradford & Seymour 1985), decrease yolk conversion efficiency (Hamor & Garside 1977; Diez & Davenport 1990) reduce development rates and delay hatching times (Mills & Barnhart 1999), but also induce premature hatching (DiMichele & Taylor 1980; Latham & Just 1989; Mills & Barnhart 1999), cause deformities (Alderdice *et al.* 1958), decrease size at hatching and reduce post-hatching success (Mills & Barnhart 1999; Richmond *et al.* 2006).

Perhaps unsurprisingly, parental care in many fish species has been reported to not only protect the embryos from predators (Sargent 1997), or to keep the eggs clean to prevent diseases (Keenleyside 1991), but also to improve the access of oxygen to embryos through a great variety of strategies (van Iersel 1953; Sevenster 1961; Wootton 1990; Green & McCormick 2005). For instance, in the hornyhead chub (*Nocomis biguttatus*), males construct nests in areas with relatively high water flow to maintain high oxygen conditions inside them (Wisenden *et al.* 2009). Under hypoxia, common and sand goby males (*Pomatoschistus microps* and *P. minutus*, respectively) build nests with wider openings (Jones & Reynolds 1999a; Lissåker & Kvarnemo 2006), and also increase their fanning frequency and duration under low oxygen conditions (Jones & Reynolds 1999b; Green & McCormick 2005; Lindström *et al.* 2006; Lissåker & Kvarnemo 2006), which improves the amount of oxygen in the close vicinity of the embryos (Green & McCormick 2005). *Amphiprion melanopus* males increase their fanning effort during brooding by up to 70%, parallel with increasing embryo metabolic demands (Green 2004). In the beaugregory damselfish (*Stegastes leucostictus*), partial cannibalism in low oxygen conditions is thought to be an adaptive method used by caring males to reduce the overall requirements of the brood, thereby ensuring the successful hatching of the uneaten eggs (Payne *et al.* 2002).

Table 1. Descriptive information of the five pipefish species studied in this thesis. Body length and egg diameter values are calculated from data presented in Papers IV and V. Picture of *S. acus* taken from <http://en.academic.ru/dic.nsf/enwiki/6676603> (8th August, 2010). All other pictures are taken by Kentaroo Tryman.

Species	<i>Syngnathus acus</i>	<i>Syngnathus typhle</i>	<i>Syngnathus rostellatus</i>	<i>Nerophis ophidion</i>	<i>Entelurus aequoreus</i>
Body length (mm)					
Female	346.3 ± 11.8	187.4 ± 5.9	119.6 ± 2.7	247.1 ± 5.5	379.8 ± 15.9
Male	346.0 ± 32.0	177.2 ± 2.5	111.8 ± 1.1	176.5 ± 2.4	298.7 ± 5.0
Brood pouch	Present	Present	Present	Absent	Absent
Egg production	Continuous	Continuous	Continuous	In clutches	In clutches
Egg size (mm)	2.44 ± 0.03	1.82 ± 0.03	1.18 ± 0.03	0.96 ± 0.03	1.03 ± 0.03
Mating system	Polygynandry	Polygynandry	Polygynandry	Polyandry	Polyandry
Sex-role	Reversed	Reversed	Reversed	Reversed	Reversed
Habitat	Among brown algae, eelgrass and sandy bottoms	Eelgrass meadows	Sandy and shallow eelgrass meadows	Eelgrass meadows	Among brown algae and eelgrass meadows



Breeding pairs of the long-finned goby (*Valenciennea longipinnis*) share the costs of oxygen provisioning as females build mounds of rubble on one of the burrow openings to enhance the flow of oxygen-rich water inside the nest, which alleviates the male's burden of fanning the eggs (Takegaki & Nakazono 1999; Takegaki 2001, 2003). In syngnathids, males of species that have brood pouches are assumed to transfer oxygen to their offspring through the vascularised skin of the brood pouch (Berglund *et al.* 1986a; Carcupino *et al.* 2002; Ripley *et al.* 2010).

Given the close link between parental care, particularly oxygenation of developing embryos, and egg size, Kolm & Ahnesjö (2005) proposed that parental care in fish may have evolved, at least partially, to buffer developing embryos from low dissolved oxygen conditions, which in turn, may have promoted the evolution of larger eggs in species with parental care.

The family Syngnathidae

The Syngnathidae (seahorses and pipefishes) is a mostly marine fish family found worldwide in warm-temperate to tropical seas, often in association with seagrasses and brown algae. All species show parental care, with males brooding the developing embryos on their body from mating to birth (Wilson *et al.* 2001). The brooding structures follow a complexity gradient ranging from a complete lack of pouch, in which the eggs are attached to the male's tail or abdomen, through partly enclosed pouches consisting of pouch plates, to fully enclosed pouches formed by two skin folds that seal in the middle during brooding, and the sac-like fully-closed pouch of seahorses (Herald 1959; Dawson 1985; Wilson *et al.* 2001; Carcupino *et al.* 2002; Wilson *et al.* 2003; Stölting & Wilson 2007).

The evolution of the syngnathid brood pouch has commonly been thought to facilitate or improve parental care. First of all, the brood pouch provides physical protection to the developing embryos from the surrounding environment. Secondly, hiding the eggs within the pouch may reduce both egg and adult predation due to improved crypsis (Svensson 1988). Thirdly, brood pouches enable a greater provision of nutrients to the developing embryos (Berglund *et al.* 1986a), as the ability of males to provide nutrients to the developing young has been shown in a number of pipefish species with brood pouches (Ripley & Foran 2006, 2009, C. Kvarnemo, K. B. Mobley, C. Partridge, A. G. Jones & I. Ahnesjö 2003–2004, unpublished data). Similarly, by sealing off the embryos from the surrounding water conditions, the brood pouch allows males to osmoregulate the embryos throughout development and also to remove waste products from embryo metabolism. This role of the brood pouch as an osmoregulatory organ is well established (Quast & Howe 1980; Carcupino *et al.* 1997; Partridge *et al.* 2007; Ripley 2009). Lastly, the brood pouch is also thought to enable males to oxygenate the embryos given the closed environment of the pouch. However, oxygenation during brooding, despite logically necessary, has received much less attention and, thus, the ability of males to maintain high within-pouch oxygen levels throughout the pregnancy has remained curiously unexplored (Carcupino *et al.* 2002; Monteiro *et al.* 2005; Ripley *et al.* 2010).

Within the brood pouch, not all eggs are always fertilized, and among the ones that are,

some offspring are not able to thrive and develop successfully (*i.e.* eggs remain undeveloped, underdeveloped or disappear with time). Thus, the number of newborn that leave the pouch at birth is frequently lower than the number of eggs transferred into the pouch. The difference between number of initial eggs and number of resulting offspring is termed brood reduction. This definition initially focused on birds and had an adaptive connotation (Lack 1966), but has later been broadened to include other taxa and relaxed from its adaptive connotation (Ahnesjö 1992b; Mock 1994). In pipefish, brood reduction is thought to occur throughout the whole brooding period and not be restricted to a particular phase (Ahnesjö 1992a, b). The potentially adaptive value of brood reduction in pipefishes has just begun to be understood (Partridge *et al.* 2009; Paczolt & Jones 2010; Sagebakken *et al.* 2010).

The extensive paternal care provided by males of many syngnathid species, irrespective of presence of brood pouches, has substantially restricted male potential reproductive rates compared to those of females, as is the case in at least two of the species studied in this thesis, *S. typhle* and *N. ophidion* (Berglund *et al.* 1989; Table 1). As a consequence, females are able to produce more eggs than males can care for during the time period of a male pregnancy and so females compete for male partners a situation termed sex-role reversal and males are the choosier sex, (Berglund *et al.* 1986a; Ahnesjö 1989; Berglund *et al.* 1989; Vincent *et al.* 1992, reviewed in Berglund & Rosenqvist 2003). Male choice can occur, both prior to and following mating. New studies have recently shown that the brood pouch in pipefishes not only functions as a tool to provide care, but also functions as a tool to optimise male reproductive output. Males of the species *Syngnathus scovelli* are able to exert cryptic choice as they show higher levels of brood reduction when mated to lower quality females, thus saving crucial resources that subsequently can be used to nurse embryos from higher quality partners (Paczolt & Jones 2010). Furthermore, brooding *S. typhle* males are able to absorb nutrients that originate from embryos during brooding (Sagebakken *et al.* 2010). Thus, brood pouches enable males to provide more extensive care to the developing offspring, but they also may provide males with a means to influence how many and which embryos to care for.

As far as we know, pipefish males of species that do not have pouches seem to receive all their eggs from one female per brooding event (Vincent *et al.* 1995; McCoy *et al.* 2001; Avise *et al.* 2002). In polygamous pipefishes that have brood pouches, however, males typically mate with several females, both within and between broods, and females mate with several males (e.g. *Syngnathus typhle*, *S. acus*, *S. rostellatus* and *S. floridae*, Table 1, Berglund *et al.* 1989; Jones *et al.* 1999). Thus, although not all syngnathid species with brood pouches mate polygynandrously (e.g. seahorses and *Syngnathus scovelli*, Jones *et al.* 2001) the evolution of brood pouches provided the opportunity for males to mate with several females. Accordingly, females of polygynandrous species produce and mature eggs continuously (Wallace & Selman 1981; Begovac & Wallace 1987) whilst females of polyandrous species that do not have brood pouches mature eggs in clutches, similarly to monogamous species (Sogabe *et al.* 2008).

Overall, the family Syngnathidae presents a great diversity in terms of ecology, type of parental care provided, egg size variation, mating systems and sex-roles. Here, I have taken

advantage of this fascinating system and of the extensive research that has already been done and that provided some of the knowledge required, to approach questions that in a number of different ways relate to egg size.

AIMS

The specific aims of this thesis were to:

1. Assess female reproductive allocations in relation to female choice and paternal quality in *Syngnathus typhle* (Papers **I** and **II**), to explore female and male parental effects in relation to compensation and differential allocation theories;
2. Explore whether, in *S. typhle*, embryos from large eggs have greater difficulties acquiring the necessary oxygen for their development and hence, are more susceptible to hypoxia (Paper **III**), and hence test the hypothesis that oxygen availability is important in constraining egg size evolution in a species with parental care;
3. Understand male parental behaviour in relation to ambient oxygen availability, egg size and presence or absence of brood pouch in pipefishes, using as study species two species with brood pouches (*Syngnathus rostellatus* and *S. typhle*) and two species that do not have brood pouches (*Entelurus aequoreus* and *Nerophis ophidion*), in a within-species and a between-species comparison (Papers **III** and **V**);
4. Understand the role of the brood pouch as an oxygen-providing structure to the developing embryos, and how presence or absence of brood pouches (between species) affect embryo growth and survival under normal and low oxygen conditions (Papers **IV** and **V**);
5. Relate presence or absence of brood pouch with egg size variation within species and average egg size between species in *Syngnathus acus*, *S. typhle*, *S. rostellatus*, *E. aequoreus* and *N. ophidion* (Papers **III** and **V**).

METHODOLOGY

Experimental fish and breeding setups

All studies were carried out at The Sven Lovén Center for Marine Sciences, Kristineberg (58°15'N, 11°28'E), in Sweden, between May and July of 2006 through to 2009. At the onset of breeding season, specimens of *Syngnathus typhle* Linnaeus, *S. acus* L., *S. rostellatus* Nilsson, *Nerophis ophidion* L. and *Entelurus aequoreus* L., were caught with a beam trawl, pulled behind a boat, in eelgrass (*Zostera marina*) meadows in shallow waters (1-6 meters) nearby the research station.

The focus of each study dictated the size classes chosen for each species and sex, and also the breeding method used. In Papers **I** and **II**, *S. typhle* males were separated by size because focus was on female reproductive allocations in relation to male size and also on male parental quality in relation to body size. Females were chosen to be of large sizes because large females produce significantly more eggs than small females do (Berglund & Rosenqvist 1990), and were thus able to mate with two males within a relatively short period of time. In these studies, it was important that each male mated with one female only and that each female mated with both a small and a large male. Thus, individual matings were done sequentially and in separate aquaria.

In Paper **III**, females were separated into small and large size classes because egg size was an important factor in the study, and egg size correlates positively with female body size in *S. typhle* (Berglund *et al.* 1986b). Males were chosen to be of intermediate sizes and within a short range of lengths in order to minimise confounding effects from variation in male parenting quality and also from differential female reproductive allocations resulting from female choice. Because the focus was on egg size rather than on partner identity, the breeding set-up allowed males to mate with multiple females of either large or small sizes. This way we obtained two groups of males: one brooding small eggs (*S. typhle* s) and the other brooding large eggs (*S. typhle* l). Similarly, in Paper **IV** males could mate multiply with large females, in order to ensure that all males brooded large eggs. Large egg size was chosen because it should elicit the greatest response from males, if embryos from large eggs consume more oxygen than embryos from small eggs. In addition, the standardisation of egg size minimised other potential sources of variation. A range of male sizes was chosen, in order to assess how the ability to oxygenate the developing embryos during brooding correlates with male size, in both high and low oxygen conditions.

In Paper **V**, two species with brood pouches (*S. typhle* and *S. rostellatus*) and two species without brood pouches (*E. aequoreus* and *N. ophidion*) were used. All sizes were chosen for both sexes in *S. rostellatus*, *N. ophidion* and *E. aequoreus*. The aim of the study was to simulate natural conditions; hence males and females were placed together in groups and allowed to mate freely. In addition, *S. typhle* was represented by data collected in Paper **III**. For the analysis, species were grouped either in relation to presence or absence of brood pouch or in reference to the size of the eggs they produced. In the egg size analysis, species groups that produce eggs larger

than 1.5mm were grouped into a large egg size group (*S. typhle* s and *S. typhle* l) and species that produce eggs smaller than 1.5mm in diameter were grouped into small egg size group (*S. rostellatus*, *N. ophidion* and *E. aequoreus*). Classification of the species as small- or large-egg-producing was done by assessing egg size beforehand, through female ovary dissections, in which a fifth species was also included, *S. acus*. However, *S. acus* could not be used in the main study due to too low sample sizes during the years when the study was performed.

Oxygen treatments and measurements

When oxygen level was manipulated, a high and a low level of 100% and 40% dissolved oxygen concentration were used, respectively. The hypoxic level of 40% O₂ was chosen based on a study on sand gobies (Lissåker *et al.* 2003) in which 35-40% O₂ was used. This value was low enough to elicit behavioural changes in male sand gobies without causing adult mortality.

Oxygen concentrations in experimental aquaria were lowered by pumping nitrogen gas into the water with the use of a MiniModule 1.7* 5.5 Membrane Contactor (Liqui-Cel, Celgard, Inc, North Carolina, USA). The unit functions as a counter-current flow system. Thus, nitrogen flows in the opposite direction to the water, continuously removing oxygen from the incoming water and replacing it with nitrogen. With this technique, we could maintain flowing water in both high and low oxygen treatment aquaria. The high oxygen level was maintained by bubbling air into the water using air-stones. Oxygen levels were measured daily with a portable oxygen meter (Handy Delta, OxyGuard, Tekno Trading AB, Säffle, Sweden) and readjusted whenever necessary (Papers III, IV and V).

In Paper IV, intra-pouch oxygen measurements were taken with a Foxy-AL300-AF probe, connected to a laptop through a MFPP100-1 oxygen sensor (OOISENSORS, Ocean Optics, B.V., Duiven, The Netherlands). During measurements, each brooding male was placed in a silicon tube filled with water from the experimental aquaria in order to keep the surrounding oxygen levels similar to the experimental conditions. The needle from the oxygen probe was inserted where the two brood pouch flaps are grown together and kept in place until three measurements were recorded within a one percentage range of oxygen concentration. This is a novel technique that enabled the direct assessment of brooding males' within-pouch oxygen concentrations throughout the pouch and also enabled assessment of concentration changes over the brooding period.

Behavioural recordings

Low levels of dissolved oxygen concentrations in the water have known effects on adult fish behaviour and physiology, which depend on the severity and duration of the hypoxic event (Kramer 1987; Pollock *et al.* 2007). These effects may include an increase in ventilation rates, aquatic-surface respiration, reduction in metabolic rate and reduction in activity. In Papers III and V, I recorded the behaviour of all experimental males during the brooding period. Specifically, I observed male ventilation rates, proportion of time spent swimming and proportion of time

spent in the upper part of the aquarium, in order to assess the effect of hypoxia on male activity patterns during brooding. To assess proportion of time spent in the upper part of the water column, the middle height of the water column was marked on the sides of each aquarium.

On days 1, 9 and 18 of the brooding period, ventilation rates and 10-minute video recordings were conducted for each male with the use of a video camera (SONY, model DCR-TRV30E). Ventilation rates (ventilations minute^{-1}) were observed directly for each individual, by counting the frequency of operculum movements for 30 seconds. The video-recordings were analysed using JWatcher v1.0 in order to extract the behaviours described above.

Statistical methods

Behavioural ecologists and biologists rarely have the privilege of presenting studies based on large sample sizes, particularly if working with large species such as mammals, many birds, reptiles and fish. As a consequence, often data are too few and treatments easily have unbalanced sample sizes, making it difficult to assess data distributions and the fitting of parametric assumptions. Even when parametric tests can be used, the resulting power of a test may be too low to provide clear confidence on the results obtained. For these reasons, the PERMANOVA+ add-on for PRIMER v6 (PRIMER-E, Plymouth, UK) was used to analyse much of the data collected for this thesis (Papers **III**, **IV** and **V**).

The rationale behind the tests in PERMANOVA+ combines parametric and non-parametric methods. PERMANOVA+ tests are based on resemblance measures (a distance, similarity or dissimilarity), commonly used in population ecology. Similar to non-parametric statistics, PERMANOVA+ tests make no explicit assumptions regarding the distributions of the original data. In similarity with parametric statistics, however, in PERMANOVA+, the values of the resemblance matrix chosen for a particular analysis are maintained, rather than ranked, and thus, they are used to assess the variability of the data, which enables the partition of multivariate variation according to more complex experimental designs and the test of interactions. In addition to these methodological steps, instead of relying on tables, PERMANOVA+ calculates the distribution of the data from the resemblance matrix chosen and tests hypotheses using permutations to obtain the relevant p-values (Anderson 2001a, b; Anderson *et al.* 2008).

In this thesis, Permdisp and Permanova routines were used to analyse univariate and multivariate data. The Permanova routine was used to analyse univariate and multivariate data in factorial, nested and repeated-measures designs, with fixed or mixed effects models, to analyse the effect of covariates and to perform pair-wise analyses. Because Permanova is susceptible to differences in the dispersion of data, in similarity to the susceptibility of Anova and Manova to heterogeneity of variances, I used Permdisp to test the homogeneity of dispersions in order to tear apart significant differences in the dispersions of the data clouds, from differences in the locations of their means.

Given the nature of the data (lengths, proportions, counts, etc), Euclidian distance-matrices (distances measured as the root of square differences between coordinates of a pair of objects)

were made prior to the analyses. In addition, multivariate data-sets were normalised (each variable had its mean subtracted and was divided by its standard deviation) to achieve a common scale.

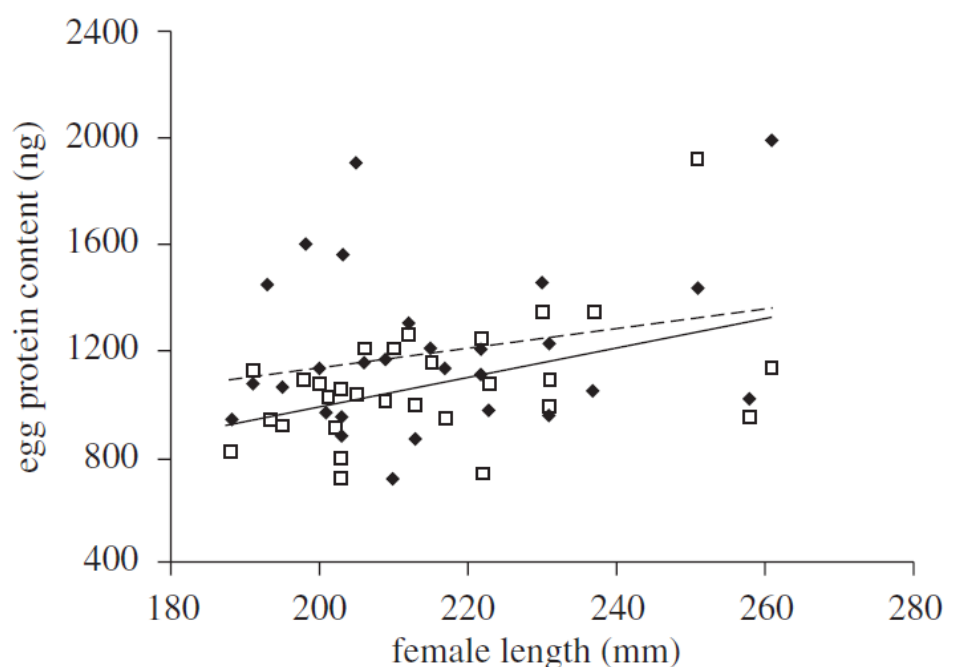
Analyses involving covariates were conducted using type I sums of squares, whilst the remaining analyses were done using type III sums of squares. The following options were chosen for all analyses: fixed effects sum to zero, model: permutation of residuals under a reduced model, number of permutations: 9999, with the exception of unifactorial Permanova analyses, in which permutation of raw data was used to calculate the significance of the model. Significance level for all tests was set at $p < 0.05$, except for removal of non-significant interaction terms, when it was set at, minimum, $p < 0.15$.

RESULTS AND DISCUSSION

Maternal effects and paternal quality

In the population of *Syngnathus typhle* studied in this thesis, females show a strong preference for large mates (Berglund *et al.* 1986b; Berglund & Rosenqvist 1993, 2003), which are known to give birth to larger offspring (Ahnesjö 1992b) and to invest more energy per offspring during brooding (Berglund *et al.* 1986b). Female potential reproductive rate is limited by male reproductive rate and female-female competition can constrain a female's ability to mate with good quality mates (Berglund *et al.* 1989; Berglund & Rosenqvist 1990; Vincent *et al.* 1994). In addition, in this population, females can be both ecologically (predation risk, absence of receptive males) and socially (female-female competition) constrained from finding preferred partners and may instead opt for mating with lower quality, smaller males, which become

Figure 1. Average protein content (nanograms per egg) of eggs provided to smaller (filled diamonds, semidotted line) and larger males (open squares, continuous line). Protein content of eggs is significantly and positively correlated to female length. $n = 58$ (from Braga Goncalves *et al.* 2010).



reproductively mature slightly later in the breeding season than larger males. Results in Paper I suggest that females reproductively compensate when they mate with smaller males, by investing (11%) more proteins into the eggs, compared to when the same females mate with larger, more preferred males (Figure 1).

However, do large males provide substantially better care than smaller males? The results from Papers II and IV, as well as taking into account the results obtained in Paper I, suggest that might be the case. Neither embryo survival nor size differed significantly between large and small males, in Paper II. However, if paternal quality is independent of male size one should expect the more protein-rich embryos developing in small males' pouches to become larger than embryos developing in the pouches of large males, given that males mated with females of similar sizes (*i.e.*, same egg size) and that females invest more proteins in eggs transferred to smaller males (Paper I). The fact that embryos brooded by large males were just as developed as embryos brooded by small males, despite the initial difference in protein concentration, suggests that large males do provide better quality care to their offspring when compared to smaller males (Paper II). This is also what the reproductive compensation hypothesis predicts, as the compensation should aim at outweighing the difference in the resulting offspring fitness between mating with a preferred and a non-preferred mate (Gowaty 2008). In addition, in Paper IV, we found that within-pouch oxygen levels during brooding are significantly and positively affected by male size, independent of the oxygen treatment they were held in during brooding.

Interestingly, Paper II also highlights the possibility that *S. typhle* females may suffer from egg overripening (Koya *et al.* 1994; Gaudemar & Beall 2005; Bromage *et al.* 2007) when prevented from breeding for extended periods of time. The females' first mating partners showed significantly higher brood reduction, independently of male size, compared to their second partners, suggesting that overripening of eggs may be a reality in this species. Importantly, the risk of overripening may further constrain females to mate with less preferred partners and put them in a situation when reproductive compensation for their partners' lower quality becomes a better solution than waiting for a better partner.

In conclusion, in this *S. typhle* population, females prefer to mate with larger males, not only because they have larger pouches, and thus, can brood more eggs, but also because larger males provide better quality care through a greater investment per offspring (Berglund *et al.* 1986b, Paper II) and better oxygenation in the pouch (Paper IV). However, females in this population are constrained by a low availability of receptive, high quality, large males during the breeding season (Berglund & Rosenqvist 1993, 2003). Thus, they are able to reproductively compensate for their partner's lower parental quality, when having to mate with smaller males (Paper I, Gowaty 2008). Given that females may reproductively compensate when their mate choice is constrained, these results leave open the possibility that small males may reduce their parental investment and canalize resources into growth to improve their future reproductive output.

Paternal behaviour in relation to oxygen availability, egg size and brood pouch presence

The social context and reproductive status of individuals can significantly affect their metabolism and, consequently, their ventilation rates (Hopkins *et al.* 1995; Masonjones 2001). In the seahorse, *Hippocampus zosterae*, males increase their oxygen consumption during the brooding period by up to 50% compared to when not brooding, although only 0.5 to 16% of this increase is due to embryo respiration (Masonjones 2001). Thus, brooding is clearly metabolically demanding for the male and his brooding capacity might be reduced under hypoxic conditions.

An increase in ventilation rate is one of the first responses of fish to low environmental oxygen conditions because it enables individuals to acquire similar amounts of oxygen per unit of time as under normal oxygen conditions, albeit at a higher energetic cost (Hughes 1973; Kramer 1987). The syngnathid species studied in this thesis significantly increased their ventilation rates when brooding under low oxygen conditions, and also over the brooding period (Paper V), and in *S. typhle* these hypoxic conditions also caused a reduction in male condition (Paper IV). This confirms that the 40% O₂ level chosen in the studies was low enough to cause hypoxia (Pollock *et al.* 2007). In addition, the four syngnathid species that were compared differed significantly in their ventilation rates, with species that brood smaller eggs breathing significantly faster than species that brood large eggs. Similarly, species that do not have brood pouches breathed significantly faster than species that have brood pouches.

In terms of behaviour, I found that although the proportion of time spent swimming was generally low and unaffected by the environmental oxygen conditions, the proportion of time the different species spent in the top half of the aquaria increased in hypoxic conditions (Paper V). Between species, fish in the large egg group swam significantly less and had lower ventilation rates than the remaining species, but they spent a similar proportion of time close to the surface as the species brooding smaller eggs (Paper V). This difference in swimming activity between species that brood large and small eggs under hypoxia, differs from the results obtained in Paper III, where *S. typhle* males brooding larger eggs were significantly more active than males brooding smaller eggs. The result obtained in Paper III was contrary to expectations because a decrease in activity levels in species that brood large eggs is expected to be an adaptive behaviour to reduce oxygen consumption by the caring male.

Species that do not have brood pouches spent significantly more time close to the water surface, where the water is expected to be relatively richer in oxygen due to gas exchange between air and surface water. Thus, by spending relatively more time closer to the water surface, species that do not have brood pouches may keep their eggs surrounded by relatively oxygen-rich water, a behaviour that would be less beneficial to species that have brood pouches (Paper V). However, this simple adaptation may entail considerable costs, as males of these species would become significantly more exposed to predation both from the air and from the surrounding water.

Effects of egg size and hypoxia on embryo survival and size

In fish, large egg size has been repeatedly linked with larger offspring size (van den Berghe & Gross 1989; Ahnesjö 1992a), which in turn typically have faster growth, increased survival, and higher fitness (Bagenal 1969), when compared to smaller counterparts. However, discussions of offspring survival at the egg stage and in relation to egg size are largely neglected (but see Balon 1984; Sargent *et al.* 1987), unless when related to low oxygen conditions (van den Berghe & Gross 1989; Quinn *et al.* 1995; Einum *et al.* 2002).

In Paper III I assessed the long standing hypothesis that embryos from large eggs do worse under hypoxic conditions compared to embryos from smaller eggs, due to their unfavourable surface area : volume ratio (Rombough & Randall 1988). In *S. typhle*, large embryos have been found to “respire more than small ones, and therefore consume their energy stores at a higher rate than small embryos” (Berglund *et al.* 1986b). Therefore, my expectations were that, under hypoxia, broods that were composed of large eggs would have lower survival and greater reductions in embryo length and weight than broods composed of small eggs. Indeed, embryos brooded in hypoxic conditions were significantly smaller and lighter than embryos brooded in fully oxygenated water, but embryos from larger eggs clearly resulted in larger and heavier embryos than those developing from smaller eggs, at both oxygen levels. Thus, the effect of hypoxia was independent of egg size, that is, embryos from large eggs did not develop significantly worse than embryos from smaller eggs in males brooding in hypoxia. Therefore, the assumption that larger eggs would suffer relatively more from oxygenation problems was not supported in this study.

One explanation for these results may be that, similarly to results reported for brown trout, metabolic demands may not increase proportionally with egg size, and thus, large embryos may have relatively lower metabolic demands than smaller embryos (Einum *et al.* 2002) and cope better under low oxygen conditions. Alternatively, a reason I did not find an interaction between egg size and oxygen treatment might be if brooding males adjust the amount of oxygen provided to the embryos, sheltering them from the deleterious effects of oxygen limitations at 40% O₂. This explanation raises the question as to whether male brood pouches in syngnathids evolved, at least partially, as an adaptation to provide embryos with oxygen during development, thus enabling the evolution of larger egg sizes in species that have brood pouches. However, based on the findings from Papers IV and V, this explanation is very unlikely, as I will explain in the next section.

In Paper V I took advantage of the egg size differences that exist between the syngnathid species that commonly occur on the Swedish west coast in order to pursue the hypothesis that egg size in fish is constrained by limitations in acquiring oxygen. There was a clear effect of egg size on embryo survival, with large eggs (*i.e.* *S. typhle* l and *S. typhle* s) presenting the lowest offspring survival. This study highlights the fact that the higher instantaneous mortality generally reported in larger eggs in fishes (Balon 1984; Sargent *et al.* 1987), is not completely annulled by parental care in syngnathids. Thus, the size advantage that juveniles from larger

eggs generally enjoy may be only significant once embryos have hatched and are no longer constrained by the egg shell to acquire oxygen. However, the effects of egg size could not be properly disentangled from potential effects of the presence of brood pouch in Paper V, due to a lack of a species that produces large eggs but does not have brood pouches.

Generally, there is a demand for a better understanding of the male pregnancy in syngnathid fishes (Stölting & Wilson 2007). In particular, the male ability to oxygenate the developing embryos has been little investigated and is consequently poorly understood (Berglund *et al.* 1986a; Carcupino *et al.* 2002). Specifically, the studies presented in this thesis strongly highlight the need for more empirical tests of the “egg size-oxygenation” hypothesis, on a greater variety of aquatic organisms, particularly in other fish families, to better understand the complexity of this area of research.

Effects of brood pouch and hypoxia on embryo survival and size

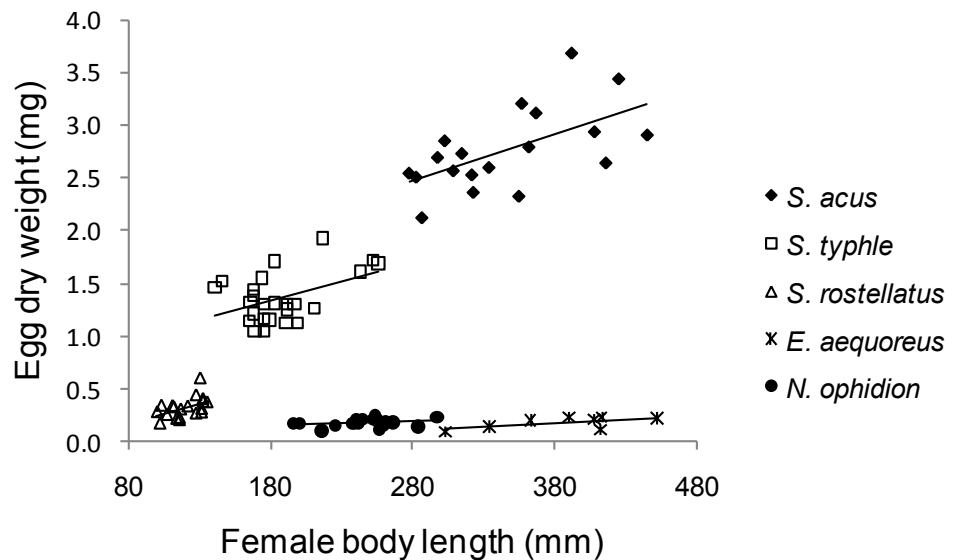
The family Syngnathidae is a fish family characterized by extensive and highly specialized paternal care, in which the males brood the eggs on their bodies throughout the developmental period. Syngnathids present a gradient in terms of paternal care provided to the embryos (Carcupino *et al.* 2002), as brood pouch complexity ranges from a complete lack of pouch in which the eggs are attached to the male’s tail or abdomen on one extreme, and the sac-like fully closed pouch of seahorses at the other extreme (Herald 1959; Dawson 1985). These different types of brood pouch have traditionally been thought to have evolved to facilitate or improve parental care (Wilson *et al.* 2001; Stölting & Wilson 2007). We know that species with and without brood pouches seem to be able to provide some nutrients to the developing embryos, although species with brood pouches are able to provide significantly more nourishment to the embryos than species that do not have brood pouches (Berglund *et al.* 1986a). In addition, only the species that possess enclosed brood pouches (e.g. *Syngnathus* spp. and seahorses) are able to osmoregulate (Haresign & Shumway 1981; Berglund *et al.* 1986a; Carcupino *et al.* 2002; Ripley & Foran 2009) and potentially provide oxygen to the offspring during brooding (Carcupino *et al.* 2002; Ripley *et al.* 2010; but see Monteiro *et al.* 2005).

In Paper V, by focusing on four pipefish species, two with fully-enclosed brood pouches and two that do not have brood pouches, I assessed how absence or presence of brood pouch relate to embryo survival and size, under high and low oxygen conditions. Against initial expectations, species with brood pouches presented lower embryo survival compared to species that do not have brood pouches, however, they were all similarly negatively affected by hypoxia. Thus, the expectations that the heavily vascularised brood pouches are adapted to supply ample oxygen to the enclosed embryos both in normoxic and in hypoxic conditions were not met. Several explanations could be discussed: First, it is difficult to conclusively disentangle the effects of egg size from those of presence of brood pouch, because *S. rostellatus* (brood pouch but small eggs) showed intermediate embryo survival compared to *S. typhle* (brood pouch and large eggs) and *N. ophidion* and *E. aequoreus* (no brood pouch and small eggs). Since there are no species

available, in the same environment, that do not have brood pouches and produce large eggs (such as for example seadragons), I could not evaluate the relative effects of both factors in the same analysis. Thus, what now appears to be a negative effect of brood pouch, could still be an effect of larger egg sizes. Second, in addition to the complexity of care that can be provided to the developing embryos, brood pouches may also give males greater influence over the number of embryos and which embryos may or may not develop (Paczolt & Jones 2010; Sagebakken *et al.* 2010). Thus, the general lower embryo survival found in species with brood pouches may result from cryptic male choice (Paczolt & Jones 2010). Third, males may not be able to maintain high intra-pouch oxygen levels throughout brooding. This possibility was tested in Paper IV. In this paper I assessed the ability of *S. typhle* males to oxygenate the developing embryos through the first 24 days of the brooding period, in both high and low ambient oxygen conditions. Males were not able to buffer embryos from environmental conditions as within-pouch oxygen levels were significantly lower in males brooding in hypoxia than in male brood pouches in fully-oxygenated water conditions. In addition, within-pouch oxygen levels were lower than those of the surrounding environment in both treatments, which highlights a possible limitation in the brooding ability of pipefish species that have brood pouches compared to species that do not have pouches. I did not measure oxygen levels in the close proximity of developing embryos from species that do not have brood pouches but, if these are similar to the surrounding water conditions, then embryos of species that do not have brood pouches seem to have access to higher oxygen concentrations in both environmental conditions. Moreover, within-pouch oxygen levels decreased throughout the brooding period, presenting a sharp decrease between days 6 and 12, irrespective of oxygen treatment. This decrease likely results from an increased demand for oxygen by the embryos as they grow and thus, have a greater consumption of oxygen (Berglund *et al.* 1986a).

Curiously, oxygen levels were highest in the bottom section of the brood pouch, where significantly larger embryos also were recorded. Due to the way eggs are transferred into a male's pouch and the possibility for males to mate with different females before the pouch becomes full with eggs (Berglund *et al.* 1988; Jones *et al.* 1999), the eggs at the bottom of the pouch are the first to be received and fertilized by the male, and arguably, the first to start to develop. This potential difference in timing of development may explain why embryos at the bottom of the pouch were larger than those in the rest of the pouch, although differences in egg densities between the different pouch sections should also be taken into consideration. The narrow bottom of the pouch usually has fewer rows of eggs and thus, embryos may have greater access to paternally derived nutrients, hormones and oxygenation. However, the positive correlation between oxygen levels and embryo length, irrespective of pouch section, suggests that, in this species, embryo growth is limited by oxygen availability and that embryos that have access to more oxygen grow faster.

Figure 2. Relationship between female length (mm) and egg dry weight (mg) for *Syngnathus acus* (N = 19), *S. typhle* (N = 25), *S. rostellatus* (N=19), *Entelurus aequoreus* (N = 9) and *Nerophis ophidion* (N = 20).



Effects of brood pouch on egg size variation within and between species

In Paper V I assessed the variation in egg size in relation to female body length within five pipefish species (*S. acus*, *S. typhle*, *S. rostellatus*, *N. ophidion* and *E. aequoreus*), and also how variation in egg size relates to presence or absence of brood pouch between species. The here investigated species with brood pouches have significantly larger and heavier eggs compared to species that do not have pouches, particularly when comparing species of similar body length ranges such as *S. acus* and *E. aequoreus*, and *S. typhle* and *N. ophidion*.

Brood pouches among the Syngnathidae have evolved towards greater complexity (Wilson *et al.* 2003), allowing a greater investment by the males (Berglund *et al.* 1986a). Thus, the egg size pattern found among these syngnathid species, with larger eggs sizes in species with brood pouches, corroborates previously documented trends between egg size and parental care in fishes (Clutton-Brock 1991; Kolm & Ahnesjö 2005). However, these results are based on too few and closely related species to be able to draw more general conclusions about the Syngnathidae family.

Egg size was positively correlated with female body length in all *Syngnathus* species, whilst *N. ophidion* and *E. aequoreus* females showed little variation in egg size, generally producing similar sized eggs, independently of their body size (Figure 2, Paper V). Within any given stable habitat, females are expected to produce eggs of one optimal size that should maximise female reproductive success. This egg size should be similar for all females, as long as all females within a population broadly experience the same environmental conditions (Smith & Fretwell 1974). However, parents seldom possess good quality information on the environments in which their offspring will develop due to temporal variation. Therefore, in variable environments, one of two strategies can be predicted: 1) selection to produce eggs of a constant size that presents the highest average fitness across offspring environments (McGinley *et al.* 1987) or, 2) a range of egg sizes within a brood that match a range of potential offspring environments (Poulin & Hamilton 2000).

The correlation between egg size and body size (between-female variation and within-female conformity) that was observed in the *Syngnathus* species does not conform to these previous predictions (between-female conformity or within-female variation), and is an area that still requires further investigation (Bernardo 1996b). If there is a tight correlation between egg size and offspring success, reduced egg size in small adults should impose a reproductive cost to those adults. A positive correlation between body and egg size could evolve when large females' offspring are more likely to experience food limitation through sibling competition (Nussbaum 1981). This should occur when large egg size increases offspring success and when large females lay more eggs in a given resource patch than small females (Bernardo 1996b). However, since small and large pipefish females are constrained by the size of the pouches of the males they mate with, for any given brood pouch, large females will transfer fewer (but larger) eggs compared to smaller females (Paper III).

Two trade-offs between egg size and egg number may be at the base of the explanation why smaller females produce small eggs in the studied *Syngnathus* spp. First, the classic trade-off in terms of female ability to produce many smaller or fewer larger eggs (Williams 1966), should impose constraints on the size of eggs females produce. However, if large egg size does not incur extra costs in terms of increased offspring mortality (Paper III), but rather improves offspring fitness (Ahnesjö 1992b), the production of large eggs should be beneficial to females of all sizes. Second, it is possible that small and large females adopt different egg size strategies according to their future mating prospects. In *S. typhle*, smaller females have greater difficulties to find mating partners, thus having lower probabilities of mating with multiple males, because males strongly prefer to mate with large females. Therefore, by producing smaller eggs small females may be able to transfer a greater number of eggs per mating event compared to if they produced larger eggs. This solution is likely to have a greater reproductive pay off to smaller females than to larger ones because larger females have less difficulties finding mating partners and (due to their larger size) are able to produce both many and large eggs, enabling them to maximise offspring fitness by producing larger eggs with more nutrients. In addition, at least in *S. typhle*, males strongly prefer to mate with large females. It is, thus, possible that small and large females adopt different egg size strategies according to their future mating prospects.

CONCLUSIONS

The evolution of the syngnathid brood pouch has been repeatedly linked to more advanced types of care because it enabled the osmoregulation (Quast & Howe 1980; Berglund *et al.* 1986a; Carcupino *et al.* 1997, 2002; Partridge *et al.* 2007), and provision of nourishment to the developing young (Haresign & Shumway 1981; Ripley & Foran 2006, 2009). However, in terms of oxygenation, the brood pouch appears to limit embryo access to oxygen and thus, limit their development (Paper IV). These findings may provide an explanation to the generally lower embryo survival observed in pipefish species with brood pouches compared to species

that do not have pouches (Paper V). Importantly, these results rule out the possibility that was proposed in Paper III, that active male oxygenation of the embryos has promoted the evolution of larger eggs in syngnathids that have enclosed brood pouches, such as *Syngnathus* species and seahorses.

In general, species with and without brood pouches seem to have developed different life history strategies. The studies in this thesis indicate that, in species that do not have brood pouches, females produce smaller eggs (Paper V) that are transferred onto a male's trunk. Because males do not brood the eggs in a pouch, females are not limited in terms of space where to transfer the eggs, and there is no or little selection for larger male sizes in these species (Berglund *et al.* 1986b; but see Monteiro *et al.* 2005). However, the absence of a brood pouch may provide less scope for substantial differences in quality of care to arise between males.

In contrast, in this study, species that have brood pouches produce larger eggs, with egg size being positively correlated to female size within species (Paper V). The extent of care (osmoregulation, oxygenation and nourishment) provided by the males and the capacity of males to influence how many offspring should develop, including the ability to absorb nutrients that originate in the eggs (Sagebakken *et al.* 2010), create a scope for notable differences in the quality of care between males. This variation in care makes the ability of females to assess male quality particularly important. In *S. typhle*, small males provide lower quality care, both in terms of investment per offspring (Berglund *et al.* 1986b; Paper II) and in terms of the amount of oxygen available to the developing embryos in the pouch (Paper IV). The quality of care provided by males may also impose strong selective pressure on egg characteristics, such as size and protein content (Paper I), in relation to the mating strategies of females of different size classes.

Where to go from here?

This thesis has improved the current knowledge on syngnathid reproductive ecology and biology, particularly on extent of paternal care in relation to egg size and presence or absence of brood pouch, and provided insights into female choice and reproductive allocations in this fish family. However, it has also raised many new and interesting questions, particularly within the following subjects:

- 1 It is clear that embryo oxygenation in aquatic environments and its relationship with egg size and parental care still requires both more empirical and theoretical work. A greater number of fish species from a variety of fish families should be studied. In particular, within- and between-species variation in timing of hatching in relation to amount of yolk left (*i.e.* inactive mass within the egg) may produce differential effects of egg size on embryo oxygenation and thus, on embryo development and survival. For example, species in which most of the yolk is absorbed before hatching may show more pronounced effects of egg size on embryo oxygenation and development, since most of the mass within the egg is active (*i.e.* consumes oxygen), than species in which larvae hatch with large yolk-sacs still attached to them.

- 2 The ability of female *S. typhle* to adjust egg protein levels in response to partner quality provides an important puzzle piece to the reproductive ecology of this species. However, it also generates a plethora of questions including: How does timing within the reproductive season, and thus short-term future reproductive prospects, influence female reproductive allocations? If large and small females adopt different breeding strategies as a reflection of their mating prospects, do small females reproductively compensate or do they adopt positive differential allocations? Can small males take advantage of female reproductive compensations and reduce their paternal investment and canalise resources into growth, thus improving their future reproductive output? More broadly, when and how are maternal effects selected for in syngnathids? Which parental effects result in sexual conflicts and what sort of sexual conflicts are in operation in syngnathids?
- 3 Related to point 2, recent studies have shown a potential for post-mating sexual selection to operate in the form of cryptic male choice, during brooding, in some *Syngnathus* species (Partridge *et al.* 2009; Paczolt & Jones 2010; Sagebakken *et al.* 2010). This promising area of research is still in its early stages and should be encouraged in order to improve our knowledge on the mechanisms and to what extent males are able to influence their post-mating reproductive success.
- 4 Male oxygenation of the developing young in syngnathid species that have brood pouches is a necessity given the closed environment of the pouch. Thus, I hypothesized whether the ability to oxygenate the embryos, protecting them from environmental variation, could have been a driving force in the evolution of brood pouch complexity. The results presented in Paper IV suggest rather that within-pouch oxygenation may be a cost, possibly traded-off against the ability to protect, nourish and osmoregulate the developing embryos in the pouch. However, this is a subject that requires a great deal of research, particularly in species with different pouch types, such as seahorses, and selected to be phylogenetically dispersed and representative for the family.
- 5 This thesis demonstrates a relationship between brood pouch complexity and increasing egg size but a more thorough analysis should be done on a greater number of species, preferably also including representatives with large eggs size but no brood pouch. It has been suggested that the evolution of brood pouches enabled males to brood a greater number of eggs, because the pouch allows eggs to be cared for in multiple layers rather than in a single layer, typical of species that do not have brood pouches (Monteiro *et al.* 2005). Together, our results suggest that, between species, the evolution of brood pouches has enabled the evolution of both larger and more eggs, although within-species trade-offs between egg number and size can still be important, as reported in this thesis. I suggest that future analyses of egg size and number in relation to brooding structure, should control for male body weight and phylogenetic proximity between species, providing a more powerful understanding of how these factors have coevolved within the family Syngnathidae.

ACKNOWLEDGEMENTS

My time as a PhD student has been a wonderful one. In the last 4.5 years I have made good friends and have many unforgettable moments that enriched my life a million-fold and that I will carry with me wherever I may head next. This PhD gave me the opportunity to travel the world and attend conferences/workshops in fantastic places such as France, Australia, USA, Réunion (admittedly France again but very, very different!), England and Norway. It also (surprisingly) allowed me some spare time to explore (small corners of) Brazil, France, Italy, Slovenia, Greece, Morocco, Norway, Canada, Denmark, Germany, Mozambique and South Africa, for which I am really grateful.

My first votes of gratitude go to the pipefish! No one can possibly imagine what you have put up with from me in the last years! From trawling you out of your habitat, to scratching your bellies in search of early pouches, watching you mate, blinding you with light torches, poking you, killing and dissecting you, drying you hard, or stealing your livers and/or embryos, dissecting those, taking pictures of them, drying them as well... and when you think that nothing else can happen to you, Olga walks into the “confocal room” and shouts “Oh Inês! That’s disgusting! They look like ugly aliens!” Indeed, you’ve even been offended... after dead! But admittedly, you’ve had your revenge, a variety of diseases and suicidal jumps from the barrels and aquaria come to mind...

I have learnt, grown and changed much during this time. For this, I owe my deepest and most sincere gratitude to my supervisors: Lotta Kvarnemo and Ingrid Ahnesjö. Thank you so much for your patience, all your help and support, and your confidence in me all the way through this process: you have been truly inspirational! I must also thank all senior and junior members of Zoologen who have helped me become a better scientist, a better researcher and a better teacher. Torgny Bohlin started off as my examiner, a role later taken over by Staffan Andersson, thank you both for your help and care. Thank you Malte Andersson, my in-house co-supervisor, for your attention and care, and always enlightening discussions about all interesting matters. Thank you, Michael Axelsson, for your help deciphering the instructions manual and for your expertise in getting our new oxygen probe to work. This thesis would not have been complete without that study and thus, without your help. Also, this thesis would have been much reduced without the help of Mattias Söderpalm (my very own PC-angel!) who, amongst many other things, rescued my work after I inadvertently erased the first three years of my research!

I would like to take this opportunity to thank Ian Fleming for accepting to be my opponent. I am sure our discussion will be challenging and very interesting. In case I am too nervous to remember to say it then, I’d like to say it now: Thank you Ian, I had a great time!

Jakob, my office colleague, thank you for all the technical help with gobys set-ups that unfortunately didn’t make it to this thesis, and for all the chocolates and croissants that always cheered me up and helped me regain the energy to keep on typing. You are the best office-mate anyone can ever wish for! Fredrik Palm, thank you for your friendship, your company and, motivation during this last phase of my PhD. I am here for you now, as you enter this same final phase. Fellow PhD’s and Post-Docs at Zoologen, in particular: Bart, Peter, Sofia A., Maria, Gry, Rasmus, Sofia B., Ola, Rupert, Hans, Daniel, Andreas, Albin, Lars, Fredrik J., Henrik, Lena, Jörgen, Caroline and Allen, thank you so

much for just about everything (including reading and criticizing my papers after you're done with the acknowledgements ☺)! You have helped make my life in Sweden a happy and interesting one.

A very special thank you I reserve for Jason Pienaar: thank you for your most wonderful friendship and help with all science matters, as well as sharing with me the burden of forcing people to attend seminars! I will come to visit soon, I promise. Jon Havenhand, thank you so much for your friendship and enthusiasm in my work, and all the patience required helping me understand statistics a little better; I am forever indebted to you. I am also very grateful to Marti Jane Anderson for her life-saving (and life-changing?) statistical advice when I most needed it. Jon Wright and Ani Kazem, I will be forever grateful for inspiring me to pursue a scientific career. Kenyon Mobley, it has been a huge pleasure to do science with you and have you as friend, I'm happy you haven't had enough of me yet! Can I ask for a recommendation letter? ... ☺

I have loved my days at Kristineberg (sorry! The Sven Lovén Centre for Marine Sciences - Kristineberg) from the first day I arrived. The sunny days sitting outside Mässan, staring at the beautiful bay over a cup of coffee and Kirsten and Magnus' food will forever stay with me. So I thank you all for being so welcoming and friendly, and for making me not want to go back to Gothenburg, not even after 3 months of intense field work. Thank you Marita, Jan-Olof and Gerhard: what would have been of me without your help every spring setting up equipment, shelves, filters, temperatures, lights and everything else! I am also grateful to the "other" pipefish group, particularly Nilla Rosenqvist and Anders Berglund for the support and borrowed equipment and for your fantastic organizational skills in all things BBQ/party-related. Mike Thorndike, thank you for being the best host a station can have and for taking friendly interest in my work. Thank you to everyone one else for sharing good and... well just good moments there! Particularly, I am very grateful for Mr. and Mrs. Slang, little Slang and new baby Slang's support these past years; had it not been for you I would have lived under a bridge the first few months of my PhD!

The work presented in this thesis would not have been possible without the help of our many fantastic field assistants: Loren Mendoza, Annika Dahlgren, Iris Duranovic, Malin Hällbom and Karin Olsson. You were brilliant from day one and I'll be forever grateful for your help and friendship! Thank you also Emma Martisson for doing a great job analysing all those hours of pipefish inactivity! The presentation of this thesis was greatly improved by the beautiful pipefish pictures taken by Kentaroo Tryman and by Sandi Manhão's magic touch on the cover picture. Thank you so much! Tobias Johansson, thank you for helping me keep my mental sanity during my writing period, even if through much sweat and hard work; I have really enjoyed your company and football chats these last months.

I would also like to thank the Fundação para a Ciência e Tecnologia, Portugal for awarding me the PhD-scholarship that allowed me to undertake this project. Funding was also provided by The Royal Society of Arts and Sciences in Göteborg (KVVS), Lundgrenska stiftelserna, Helge Ax:son Johnsons stiftelse and Adlerbertska stipendiefonden.

Family! Mãe, pai, João, Rita, Sérgia, Amélia, avó, tia, Luís, Miguel, Joana e Eduarda. You mean so much to me I don't know where to begin... you have always believed in me and wished nothing

but the best for me. Your unfaltering support gave me the courage to proceed and follow my dreams. I must also thank Irene and Hans, Jonatan and Tobias, Lisa, Isa and Freja, for always being so understanding when I “ruined” family gatherings with my books and papers and my laptop-driven “boring life”. Future unemployment should give me a bit more spare time...

Linus, you’re a companion in the true sense of the word. You were there on day one and you are here now. You never doubted me for a second, encouraged me all the way through and fought me when I doubted myself. How can I ever repay you? This thesis is for you.

REFERENCES

- Agrawal, A. A., Laforsch, C. & Tollrian, R. 1999. Transgenerational induction of defences in animals and plants. *Nature* **401**: 3408-3415.
- Ahnesjö, I. 1989. Sex-role reversal in two pipefish (Syngnathidae) species: paternal care and male limitation of female reproductive success. In *Department of Zoology*, PhD. Uppsala: University of Uppsala.
- Ahnesjö, I. 1992a. Consequences of male brood care - weight and number of newborn in a sex-role reversed pipefish. *Functional Ecology* **6**: 274-281.
- Ahnesjö, I. 1992b. Fewer newborn result in superior juveniles in the paternally brooding pipefish *Syngnathus typhle* L. *Journal of Fish Biology* **41**: 53-63.
- Alderdice, D. F., Wickett, W. P. & Brett, J. R. 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. *Journal Fisheries Research Board of Canada* **15**: 229-249.
- Alonzo, S. H. 2010. Social and coevolutionary feedbacks between mating and parental investment. *Trends in Ecology & Evolution* **25**: 99-108. Doi:10.1016/j.tree.2009.07.012
- Anderson, M. J. 2001a. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32-46.
- Anderson, M. J. 2001b. Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences* **58**: 626-639.
- Anderson, M. J., Gorley, R. N. & Clarke, K. R. 2008. *PERMANOVA+ for PRIMER: Guide to software and statistical methods*. Plymouth: PRIMER-E Ltd.
- Anderson, W., Kim, Y.-K. & Gowaty, P. 2007. Experimental constraints on female and male mate preferences in *Drosophila pseudoobscura* decrease offspring viability and reproductive success of breeding pairs. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 4484-4488.
- Avise, J. C., Jones, A. G., Walker, D. & DeWoody, J. A. 2002. Genetic mating systems and reproductive natural histories of fishes: Lessons for ecology and evolution. *Annual Review of Genetics* **36**: 19-45. Doi:10.1146/annurev.genet.36.030602.090831
- Bagenal, T. 1969. The relationships between food supply and fecundity in brown trout, *Salmo trutta*. *Journal of Fish Biology* **1**: 167-182.
- Bagenal, T. B. 1967. A short review of fish fecundity. In *The Biological Basis of Freshwater Fish Production* (ed. S. D. Gerking), pp. 89-111. Oxford: Blackwell Scientific.
- Balon, E. K. 1984. Patterns in the evolution of reproductive styles in fishes. In *Fish reproduction: strategies and tactics* (ed. R. J. Wootton & C. W. Potts), pp. 35-53. London: Academic Press.
- Balshine-Earn, S. 1995. The costs of parental care in Galilee St. Peter's fish, *Sarotherodon galilaeus*. *Animal Behaviour* **50**: 1-7.
- Begovac, P. C. & Wallace, R. A. 1987. Ovary of the pipefish *Syngnathus scovelli*. *Journal of Morphology* **193**: 117-133.
- Berezovsky, V., Goida, E., Mukalov, I. & Sushko, B. 1979. Experimental study of oxygen distribution

- in *Misgurnus fossilis* eggs. *Fiziologicheskii Zhurnal (kiev)* **25**: 379-389.
- Berglund, A., Rosenqvist, G. & Svensson, I. 1986a. Reversed sex-roles and parental energy investment in zygotes of two pipefish (Syngnathidae) species. *Marine Ecology-Progress Series* **29**: 209-215.
- Berglund, A., Rosenqvist, G. & Svensson, I. 1986b. Mate choice, fecundity and sexual dimorphism in two pipefish species (Syngnathidae). *Behavioral Ecology and Sociobiology* **19**: 301-307.
- Berglund, A., Rosenqvist, G. & Svensson, I. 1988. Multiple matings and paternal brood care in the pipefish *Syngnathus typhle*. *Oikos* **51**: 184-188.
- Berglund, A., Rosenqvist, G. & Svensson, I. 1989. Reproductive success of females limited by males in two pipefish species. *American Naturalist* **133**: 506-516.
- Berglund, A. & Rosenqvist, G. 1990. Male limitation of female reproductive success in a pipefish - effects of body-size differences. *Behavioral Ecology and Sociobiology* **27**: 129-133.
- Berglund, A. & Rosenqvist, G. 1993. Selective males and ardent females in pipefishes. *Behavioral Ecology and Sociobiology* **32**: 331-336.
- Berglund, A. & Rosenqvist, G. 2003. Sex role reversal in pipefish. *Advances in the Study of Behavior* **32**: 131-167.
- Bernardo, J. 1996a. Maternal effects in animal ecology. *American Zoologist* **36**: 83-105.
- Bernardo, J. 1996b. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *American Zoologist* **36**: 216-236.
- Bilton, H. T., Alderdice, D. F. & Schnute, J. T. 1982. Influence of time and size at release of juvenile coho salmon (*Onchorhynchus kisutch*) on returns at maturity. *Canadian Journal of Fisheries and Aquatic Sciences* **39**: 426-447.
- Bluhm, C. & Gowaty, P. 2004a. Social constraints on female mate preferences in mallards *Anas platyrhynchos* decrease offspring viability and mother's productivity. *Animal Behaviour* **68**: 977-983.
- Bluhm, C. K. & Gowaty, P. A. 2004b. Reproductive compensation for offspring viability deficits by female mallards, *Anas platyrhynchos*. *Animal Behaviour* **68**: 985-992.
- Bradford, D. F. & Seymour, R. S. 1985. Energy conservation during the delayed hatching period in the frog *Pseudophryne bibroni*. *Physiological Zoology* **58**: 491-496.
- Braga Goncalves, I., Mobley, K. B., Ahnesjö, I., Sagebakken, G., Jones, A. G. & Kvarnemo, C. 2010. Reproductive compensation in broad-nosed pipefish females. *Proceedings of the Royal Society of London Series B-Biological Sciences* **277**: 1581-1587. Doi:10.1098/rspb.2009.2290
- Bromage, N., Bruce, M., Basavaraja, N., Rana, K., Shields, R., Young, C., Dye, J., Smith, P., Gillespie, M. & Gamble, J. 2007. Egg quality determinants in finfish. The role of overripening with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus hippoglossus*. *Journal of the World Aquaculture Society* **25**: 13-21. Doi:10.1111/j.1749-7345.1994.tb00799.x
- Brooks, S., Tyler, C. R. & Sumpter, J. P. 1997. Quality in fish: what makes a good egg? *Reviews in Fish Biology and Fisheries* **7**: 387-416.
- Burley, N. 1986. Sexual selection for aesthetic traits in species with biparental care. *American Naturalist* **127**: 415-445.

- Burley, N. 1988. The Differential-allocation hypothesis - an experimental test. *American Naturalist* **132**: 611-628.
- Byers, J. & Waits, L. 2006. Good genes sexual selection in nature. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 16343-16345.
- Carcupino, M., Baldacci, A., Mazzini, M. & Franzoi, P. 1997. Morphological organization of the male brood pouch epithelium of *Syngnathus abaster* Risso (Teleostea, Syngnathidae) before, during, and after egg incubation. *Tissue & Cell* **29**: 21-30.
- Carcupino, M., Baldacci, A., Mazzini, M. & Franzoi, P. 2002. Functional significance of the male brood pouch in the reproductive strategies of pipefishes and seahorses: a morphological and ultrastructural comparative study on three anatomically different pouches. *Journal of Fish Biology* **61**: 1465-1480. Doi:10.1006/jfbi.2002.2160
- Chambers, R. C. & Leggett, W. C. 1996. Maternal influences on variation in egg sizes in temperate marine fishes. *American Zoologist* **36**: 180-196.
- Charnov, E. L., Turner, T. F. & Winemiller, K. O. 2001. Reproductive constraints and the evolution of life histories with indeterminate growth. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 9460-9464.
- Clutton-Brock, T. 1991. *The evolution of parental care*. Monographs in behaviour and ecology. Princeton: Princeton University Press.
- Clutton-Brock, T. H. & Vincent, A. C. J. 1991. Sexual selection and the potential reproductive rates of males and females. *Nature* **351**: 58-60.
- Clutton-Brock, T. H. & Parker, G. A. 1992. Potential reproductive rates and the operation of sexual selection. *Quarterly Review of Biology* **67**: 437-456.
- Coleman, R. M. 1992. Reproductive biology and female parental care in the cockscomb prickleback, *Anoplarchus purpureus* (Pisces, Stichaeidae). *Environmental Biology of Fishes* **35**: 177-186.
- Cooke, S. J., Schreer, J. F., Wahl, D. H. & Philipp, D. P. 2010. Cardiovascular performance of six species of field-acclimatized centrarchid sunfish during the parental care period. *Journal of Experimental Biology* **213**: 2332-2342. Doi:10.1242/jeb.030601
- Crisp, D. 1959. The rate of development of *Balanus balanoides* (L.) embryos in vitro. *Journal of Animal Ecology* **28**: 119-132.
- Darwin, C. 1859. *On the origin of species by means of natural selection*. London: Murray.
- Darwin, C. 1971. *The descent of man, and selection in relation to sex*. London: Murray.
- Davies, N. & Hatchwell, B. 1992. The value of male parental care and its influence on reproductive allocation by male and female dunnocks. *Journal of Animal Ecology* **61**: 259-272.
- Dawson, C. E. 1985. *Indo-Pacific pipefishes (Red Sea to the Americas)*. Ocean Springs, Mississippi, USA: The Gulf Coast Research Laboratory.
- Daykin, P. 1965. Application of mass transport theory to the problem of respiration of fish eggs. *Journal of the Fisheries Research Board of Canada* **22**: 159-170.
- De Martini, E. E. 1987. Paternal defense, cannibalism and polygamy - factors influencing the reproductive success of painted greenling (Pisces, Hexagrammidae). *Animal Behaviour* **35**:

1145-1158.

- Diez, J. M. & Davenport, J. 1990. Energy exchange between the yolk and embryo of dogfish (*Scyliorhinus canicula* L.) eggs held under normoxic, hypoxic and transient anoxic conditions. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* **96**: 825-830.
- DiMichele, L. & Taylor, M. 1980. The environmental control of hatching in *Fundulus heteroclitus*. *Journal of Experimental Zoology* **214**: 181-187.
- Doudoroff, P. & Shumway, D. 1970. Dissolved oxygen requirements of freshwater fishes. Fisheries Technical Paper 86: Food and Agriculture Organizations of the United Nations.
- Duarte, C. M. & Alcaraz, M. 1989. To produce many small or few large eggs - a size-independent reproductive tactic of fish. *Oecologia* **80**: 401-404.
- Einum, S., Hendry, A. P. & Fleming, I. A. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**: 2325-2330. Doi:10.1098/rspb.2002.2150
- Ernsting, G. & Isaaks, A. 2000. Ectotherms, temperature, and trade-offs: size and number of eggs in a carabid beetle. *American Naturalist* **155**: 804-813.
- Fernández, M., Pardo, L. M. & Baeza, J. A. 2002. Patterns of oxygen supply in embryo masses of brachyuran crabs throughout development: the effect of oxygen availability and chemical cues in determining female brooding behavior. *Marine Ecology-Progress Series* **245**: 181-190.
- Fisher, R. 1930. *The genetical theory of natural selection*. Oxford: Oxford University Press.
- Fowler, L. G. 1972. Growth and mortality of fingerling chinook salmon as affected by egg size. *Progressive Fish-Culturist* **34**: 66.
- Fox, C. W. & Czesak, M. E. 2000. Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology* **45**: 341-369.
- Gaudemar, B. d. & Beall, E. 2005. Effects of overripening on spawning behaviour and reproductive success of Atlantic salmon females spawning in a controlled flow channel. *Journal of Fish Biology* **53**: 434-446.
- Gonzalez-Voyer, A., Fitzpatrick, J. L. & Kolm, N. 2008. Sexual selection determines parental care patterns in cichlid fishes. *Evolution* **62**: 2015-2026.
- Goodwin, N. B., Dulvy, N. K. & Reynolds, J. D. 2002. Life-history correlates of the evolution of live bearing in fishes. *Philosophical Transactions of the Royal Society B-Biological Sciences* **357**: 259-267. Doi:10.1098/rstb.2001.0958
- Gowaty, P., Anderson, W., Bluhm, C., Drickamer, L., Kim, Y. & Moore, A. 2007. The hypothesis of reproductive compensation and its assumptions about mate preferences and offspring viability. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 15023-15027. Doi:10.1073/pnas.0706622104
- Gowaty, P. 2008. Reproductive compensation. *Journal of Evolutionary Biology* **21**: 1189-1200. Doi:10.1111/j.1420-9101.2008.01559.x
- Green, B. S. 2004. Embryogenesis and oxygen consumption in benthic egg clutches of a tropical clownfish, *Amphiprion melanopus* (Pomacentridae). *Comparative Biochemistry*

- and Physiology A-Molecular & Integrative Physiology* **138**: 33-38. Doi:10.1016/j.cbpb.2004.02.014
- Green, B. S. & McCormick, M. I. 2005. O₂ replenishment to fish nests: males adjust brood care to ambient conditions and brood development. *Behavioral Ecology* **16**: 389-397. Doi:10.1093/beheco/ari007
- Green, B. S., Anthony, K. R. N. & McCormick, M. I. 2006. Position of egg within a clutch is linked to size at hatching in a demersal tropical fish. *Journal of Experimental Marine Biology and Ecology* **329**: 144-152. Doi:10.1016/j.jembe.2005.08.012
- Gross, M. R. & Sargent, R. C. 1985. The evolution of male and female parental care in fishes. *American Zoologist* **25**: 807-822.
- Hale, R. E. & St Mary, C. M. 2007. Nest tending increases reproductive success, sometimes: environmental effects on paternal care and mate choice in flagfish. *Animal Behaviour* **74**: 577-588. Doi:10.1016/j.anbehav.2006.12.013
- Hamor, T. & Garside, E. T. 1977. Size relations and yolk utilization in embryonated ova and alevins of Atlantic salmon *Salmo salar* L. in various combinations of temperature and dissolved oxygen. *Canadian Journal of Zoology* **55**: 1892-1898.
- Haresign, T. & Shumway, S. 1981. Permeability of the marsupium of the pipefish, *Syngnathus fuscus* to (14C)-alpha amino isobutyris acid. *Comparative Biochemistry and Physiology* **69 (A)**: 603-604.
- Harris, W. E. & Uller, T. 2009. Reproductive investment when mate quality varies: differential allocation versus reproductive compensation. *Philosophical Transactions of the Royal Society B-Biological Sciences* **364**: 1039-1048. Doi:10.1098/rstb.2008.0299
- Hendry, A. P., Day, T. & Cooper, A. B. 2001. Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *The American Naturalist* **157**: 387-407.
- Hendry, A. P. & Day, T. 2003. Revisiting the positive correlation between female size and egg size. *Evolutionary Ecology and Research* **5**: 421-429.
- Henrich, S. 1988. Variation in offspring sizes of the poeciliid fish *Heterandria formosa* in relation to fitness. *Oikos* **51**: 13-18.
- Herald, E. S. 1959. From pipefish to seahorse - a study of phylogenetic relationships. *Proceedings of the California Academy of Sciences* **29**: 465-473.
- Hinton, H. 1981. *Biology of insect eggs*. Oxford: Pergamon Press.
- Hoelzer, G. 1989. The good parent process of sexual selection. *Animal Behaviour* **38**: 1067-1078.
- Holtby, L. B. & Healey, M. C. 1986. Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 1946-1959.
- Hopkins, T. E., Eldridge, M. B. & Cech, J. J. 1995. Metabolic costs of viviparity in yellowtail rockfish, *Sebastes flavidus*. *Environmental Biology of Fishes* **43**: 77-84.
- Hughes, G. M. 1973. Respiratory responses to hypoxia in fish. *American Zoologist* **13**: 475-489.
- Jensen, A. L. 1996. Beverton and Holt life history invariants result from optimal trade-off of reproduction and survival. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 820-822.

- Jones, A. G., Rosenqvist, G., Berglund, A. & Avise, J. C. 1999. The genetic mating system of a sex-role-reversed pipefish (*Syngnathus typhle*): a molecular inquiry. *Behavioral Ecology and Sociobiology* **46**: 357-365.
- Jones, J. C. & Reynolds, J. D. 1999a. Oxygen and the trade-off between egg ventilation and brood protection in the common goby. *Behaviour* **136**: 819-832.
- Jones, J. C. & Reynolds, J. D. 1999b. Costs of egg ventilation for male common gobies breeding in conditions of low dissolved oxygen. *Animal Behaviour* **57**: 181-188.
- Kamler, E. 1992. *Early life history of fish: an energetics approach*. London: Chapman and Hall.
- Kaplan, R. H. & King, E. G. 1997. Egg size is a developmentally plastic trait: evidence from long term studies in the frog *Bombina orientalis*. *Herpetologica* **53**: 149-165.
- Keenleyside, M. 1991. Parental care. In *Cichlid fishes: behaviour, ecology and evolution* (ed. M. Keenleyside). London: Chapman & Hall.
- Keister, J. E., Houde, E. D. & Breitburg, D. L. 2000. Effects of bottom-layer hypoxia on abundances and depth distributions of organisms in Patuxent river, Chesapeake Bay. *Marine Ecology Progress Series* **205**: 43-59.
- Kerfoot, W. 1974. Egg-size cycle of a cladoceran. *Ecology* **55**: 1259-1270.
- Kokko, H. & Johnstone, R. A. 2002. Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **357**: 319-330. Doi:10.1098/rstb.2001.0926
- Kokko, H. & Jennions, M. D. 2003. It takes two to tango. *Trends in Ecology & Evolution* **18**: 103-104.
- Kokko, H. & Jennions, M. D. 2008. Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology* **21**: 919-948.
- Kolm, N. & Ahnesjö, I. 2005. Do egg size and parental care coevolve in fishes? *Journal of Fish Biology* **66**: 1499-1515. Doi:10.1111/j.0022-1112.2005.00777.x
- Kolm, N., Goodwin, N. B., Balshine, S. & Reynolds, J. D. 2006. Life history evolution in cichlids 1: revisiting the evolution of life histories in relation to parental care. *Journal of Evolutionary Biology* **19**: 66-75.
- Koya, Y., Matsubara, T. & Nakagawa, T. 1994. Efficient artificial fertilization method based on the ovulation cycle in barfin flounder *Verasper moseri*. *Fisheries Science* **60**: 537-540.
- Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes* **18**: 81-92.
- Lack, D. 1966. *Population studies of birds*. Oxford: Clarendon Press.
- Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences of the United States of America* **78**: 3721-3725.
- Latham, K. E. & Just, J. J. 1989. Oxygen availability provides a signal for hatching in the rainbow trout (*Salmo gairdneri*) embryo. *Canadian Journal of Fisheries and Aquatic Sciences* **46**: 55-58.
- Lavery, R. J. & Keenleyside, M. H. A. 1990. Filial cannibalism in the biparental fish *Cichlasoma*

- nigrofasciatum* (Pisces, Cichlidae) in response to early brood reductions. *Ethology* **86**: 326-338.
- Lessells, C. M. 2006. The evolutionary outcome of sexual conflict. *Philosophical Transactions of the Royal Society B-Biological Sciences* **361**: 301-317. Doi:10.1098/rstb.2005.1795
- Lindström, K., St. Mary, C. M. & Pampoulie, C. 2006. Sexual selection for male parental care in the sand goby, *Pomatoschistus minutus*. *Behavioral Ecology and Sociobiology* **60**: 46-51.
- Lindström, K. & St. Mary, C. M. 2008. Parental care and sexual selection. In *Fish behaviour* (ed. C. Magnhagen, V. A. Braithwaite, E. Forsgren & B. G. Kapoor), pp. 377-409. Enfield, NH, USA: Science Publishers.
- Lissåker, M., Kvarnemo, C. & Svensson, O. 2003. Effects of a low oxygen environment on parental effort and filial cannibalism in the male sand goby, *Pomatoschistus minutus*. *Behavioral Ecology* **14**: 374-381.
- Lissåker, M. & Kvarnemo, C. 2006. Ventilation or nest defense - parental care trade-offs in a fish with male care. *Behavioral Ecology and Sociobiology* **60**: 864-873. Doi:10.1007/s00265-006-0230-0
- Loyau, A., Saint Jalme, M., Mauget, R. & Sorci, G. 2007. Male sexual attractiveness affects the investment of maternal resources into the eggs in peafowl (*Pavo cristatus*). *Behavioral Ecology and Sociobiology* **61**: 1043-1052. Doi:10.1007/s00265-006-0337-3
- Magee, S. E., Neff, B. D. & Knapp, R. 2006. Plasma levels of androgens and cortisol in relation to breeding behavior in parental male bluegill sunfish, *Lepomis macrochirus*. *Hormones and Behavior* **49**: 598-609. Doi:10.1016/j.yhbeh.2005.12.003
- Magnhagen, C. 1991. Predation risk as a cost of reproduction. *Trends in Ecology & Evolution* **6**: 183-185.
- Manica, A. 2003. The effect of brood size and age on partial filial cannibalism in the scissortail sergeant. *Journal of Fish Biology* **63**: 37-47. Doi:10.1046/j.1095-8649.2003.00113.x
- Manica, A. 2004. Parental fish change their cannibalistic behaviour in response to the cost-to-benefit ratio of parental care. *Animal Behaviour* **67**: 1015-1021. Doi:10.1016/j.anbehav.2003.09.011
- Mank, J. E., Promislow, D. E. L. & Avise, J. C. 2005. Phylogenetic perspectives in the evolution of parental care in ray-finned fishes. *Evolution* **59**: 1570-1578.
- Marshall, D. & Uller, T. 2007. When is a maternal effect adaptive? *Oikos* **116**: 1957-1963.
- Masonjones, H. D. 2001. The effect of social context and reproductive status on the metabolic rates of dwarf seahorses (*Hippocampus zosterae*). *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology* **129**: 541-555.
- McCormick, M. I. 2003. Consumption of coral propagules after mass spawning enhances larval quality of damselfish through maternal effects. *Oecologia* **136**: 37-45. Doi:10.1007/s00442-003-1247-y
- McCormick, M. I. & Hoey, A. S. 2004. Larval growth history determines juvenile growth and survival in a tropical marine fish. *Oikos* **106**: 225-242.
- McCoy, E. E., Jones, A. G. & Avise, J. C. 2001. The genetic mating system and tests for cuckoldry

- in a pipefish species in which males fertilize eggs and brood offspring externally. *Molecular Ecology* **10**: 1793-1800.
- McGinley, M. A., Temme, D. H. & Geber, M. A. 1987. Parental investment in offspring in variable environments - theoretical and empirical considerations. *American Naturalist* **130**: 370-398.
- Mills, N. E. & Barnhart, M. C. 1999. Effects of hypoxia on embryonic development in two *Ambystoma* and two *Rana* species. *Physiological and Biochemical Zoology* **72**: 179-188.
- Mock, D. 1994. Brood reduction: narrow sense, broad sense. *Journal of Avian Biology* **25**: 3-7.
- Monteiro, N. M., Almada, V. C. & Vieira, M. N. 2005. Implications of different brood pouch structures in syngnathid reproduction. *Journal of the Marine Biological Association of the United Kingdom* **85**: 1235-1241.
- Moore, A., Gowaty, P. & Moore, P. 2003. Females avoid manipulative males and live longer. *Journal of Evolutionary Biology* **16**: 523-530.
- Mousseau, T. A. & Dingle, H. 1991. Maternal effects in insect life histories. *Annual Review of Entomology* **36**: 511-534.
- Naylor, J. K., Taylor, E. W. & Bennett, D. B. 1999. Oxygen uptake of developing eggs of *Cancer pagurus* (Crustacea: Decapod: Cancridae) and consequent behaviour of the ovigerous females. *Journal of the Marine Biological Association of the United Kingdom* **79**: 305-315.
- Neff, B. D. 2003. Paternity and condition affect cannibalistic behavior in nest-tending bluegill sunfish. *Behavioral Ecology and Sociobiology* **54**: 377-384.
- Nussbaum, R. A. 1981. Seasonal shifts in clutch size and egg size in the side-blotched lizard, *Uta stansburiana* Baird and Girard. *Oecologia* **49**: 8-13.
- Nussbaum, R. A. 1985. The evolution of parental care in salamanders. *Miscellaneous Publications of the Museum of Zoology of the University of Michigan* **169**: 1-50.
- Nussbaum, R. A. 1987. Parental care and egg size in salamanders - an examination of the safe harbor hypothesis. *Researches on Population Ecology* **29**: 27-44.
- Nussbaum, R. A. & Schultz, D. L. 1989. Coevolution of parental care and egg size. *American Naturalist* **133**: 591-603.
- Paczolt, K. A. & Jones, A. G. 2010. Postcopulatory sexual selection and sexual conflict in the evolution of male pregnancy. *Nature* **464**: 401-404.
- Parker, G. A. 1979. Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (ed. M. S. Blum & N. A. Blum), pp. 123-166. London: Academic Press.
- Parker, G. A. & Begon, M. 1986. Optimal egg size and clutch size - effects of environment and maternal phenotype. *American Naturalist* **128**: 573-592.
- Partridge, C., Shardo, J. & Boettcher, A. 2007. Osmoregulatory role of the brood pouch in the euryhaline Gulf pipefish, *Syngnathus scovelli*. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology* **147**: 556-561. Doi:10.1016/j.cbpa.2007.02.007
- Payne, A. G., Smith, C. & Campbell, A. C. 2002. Filial cannibalism improves survival and development of beaugregory damselfish embryos. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**: 2095-2102.

- Pelster, B. 1999. Environmental influences on the development of the cardiac system in fish and amphibians. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology* **124**: 407-412.
- Pilz, K. M., Smith, H. G., Sandell, M. I. & Schwabl, H. 2003. Inter-female variation in egg yolk androgen allocation in the European starling: do high-quality females invest more? *Animal Behaviour* **65**: 841-850.
- Pollock, M. S., Clarke, L. M. J. & Dube, M. G. 2007. The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environmental Reviews* **15**: 1-14. Doi:10.1139/a06-006
- Pomiankowski, A. 1987. The costs of choice in sexual selection. *Journal of Theoretical Biology* **128**: 195-218.
- Poulin, R. & Hamilton, W. J. 2000. Egg size variation as a function of environmental variability in parasitic trematodes. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **78**: 564-569.
- Quast, W. D. & Howe, N. R. 1980. The osmotic role of the brood pouch in the pipefish *Syngnathus scovelli*. *Comparative Biochemistry and Physiology* **67**: 675-678.
- Queller, D. C. 1997. Why do females care more than males? *Proceedings of the Royal Society of London, series B* **264**: 1555-1557.
- Quinn, T. P., Hendry, A. P. & Wetzel, L. A. 1995. The influence of life history trade-offs and the size of incubation gravels on egg size variation in sockeye salmon (*Oncorhynchus nerka*). *Oikos* **74**: 425-438.
- Ratikainen, II & Kokko, H. 2010. Differential allocation and compensation: who deserves the silver spoon? *Behavioral Ecology* **21**: 195-200. Doi:10.1093/beheco/arp168
- Reardon, E. E. & Chapman, L. J. 2009. Hypoxia and life-history traits in a eurytopic African cichlid. *Journal of Fish Biology* **75**: 1795-1815. Doi:10.1111/j.1095-8649.2009.02429.x
- Reynolds, J. D. & Jones, J. C. 1999. Female preference for preferred males is reversed under low oxygen conditions in the common goby (*Pomatoschistus microps*). *Behavioral Ecology* **10**: 149-154.
- Reynolds, J. D., Goodwin, N. B. & Freckleton, R. P. 2002. Evolutionary transitions in parental care and live bearing in vertebrates. *Philosophical Transactions of the Royal Society B-Biological Sciences* **357**: 269-281. Doi:10.1098/rstb.2001.0930
- Richmond, C., Marcus, N. H., Sedlacek, C., Miller, G. A. & Oppert, C. 2006. Hypoxia and seasonal temperature: short-term effects and long-term implications for *Acartia tonsa dana*. *Journal of Experimental Marine Biology and Ecology* **328**: 177-196.
- Ripley, J. L. & Foran, C. M. 2006. Differential parental nutrient allocation in two congeneric pipefish species (Syngnathidae: *Syngnathus* spp.). *Journal of Experimental Biology* **209**: 1112-1121. Doi:10.1242/jeb.02119
- Ripley, J. L. 2009. Osmoregulatory role of the paternal brood pouch for two *Syngnathus* species. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology* **154**: 98-104. Doi:10.1016/j.cbpa.2009.05.003
- Ripley, J. L. & Foran, C. M. 2009. Direct evidence for embryonic uptake of paternally-derived

- nutrients in two pipefishes (Syngnathidae: *Syngnathus* spp.). *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **179**: 325-333. Doi:10.1007/s00360-008-0316-2
- Ripley, J. L., Williams, P. S. & Foran, C. M. 2010. Morphological and quantitative changes in paternal brood-pouch vasculature during embryonic development in two *Syngnathus* pipefishes. *Journal of Fish Biology* **77**: 67-79. Doi:10.1111/j.1095-8649.2010.02659.x
- Roff, D. 1992. *The evolution of life histories: theory and analysis*. New York: Chapman & Hall.
- Rombough, P. 1988. Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. *Canadian Journal of Zoology* **66**: 651-660.
- Rombough, P. J. & Randall, D. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In *Fish physiology - The physiology of developing fish. Part A. Eggs and Larvae*, vol. XI (ed. W. S. Hoar), pp. 59-161.
- Rombough, P. J. 2007. Oxygen as a constraining factor in egg size evolution in salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **64**: 692-699.
- Royle, N. J., Hartley, I. R. & Parker, G. A. 2002. Sexual conflict reduces offspring fitness in zebra finches. *Nature* **416**: 733-736.
- Sabat, A. M. 1994. Mating success in brood-guarding male rock bass, *Ambloplites rupestris* - the effect of body size. *Environmental Biology of Fishes* **39**: 411-415.
- Sagebakken, G., Ahnesjö, I., Mobley, K. B., Goncalves, I. B. & Kvarnemo, C. 2010. Brooding fathers, not siblings, take up nutrients from embryos. *Proceedings of the Royal Society of London Series B-Biological Sciences* **277**: 971-977. Doi:10.1098/rspb.2009.1767
- Sandvik, M., Rosenqvist, G. & Berglund, A. 2000. Male and female mate choice affects offspring quality in a sex-role-reversed pipefish. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**: 2151-2155.
- Sargent, R. C., Gross, M. R. & Vandenberghe, E. P. 1986. Male mate choice in fishes. *Animal Behaviour* **34**: 545-550.
- Sargent, R. C., Taylor, P. D. & Gross, M. R. 1987. Parental care and the evolution of egg size in fishes. *American Naturalist* **129**: 32-46.
- Sargent, R. C. 1997. Parental care. In *Behavioural Ecology of Teleost Fishes* (ed. J. Godin), pp. 292-315. Oxford: Oxford University Press.
- Sevenster, P. 1961. A causal analysis of a displacement activity (fanning in *Gasterosteus aculeatus* L.). *Behaviour (Suppl. 9)*: 1-170.
- Sheldon, B. C. 2000. Differential allocation: tests, mechanisms and implications. *Trends in Ecology & Evolution* **15**: 397-402.
- Shine, R. 1978. Propagule size and parental care: The "Safe Harbor" hypothesis. *Journal of Theoretical Biology* **75**: 417-424.
- Shine, R. 1989. Alternative models for the evolution of offspring size. *American Naturalist* **134**: 311-317.
- Smith, C. & Fretwell, S. 1974. The optimal balance between size and number of offspring. *American Naturalist* **108**: 499-506.

- Smith, C. & Wootton, R. J. 1995. The costs of parental care in teleost fishes. *Reviews in Fish Biology and Fisheries* **5**: 7-22.
- Sogabe, A., Matsumoto, K., Ohashi, M., Watanabe, A., Takata, H., Murakami, Y., Omori, K. & Yanagisawa, Y. 2008. A monogamous pipefish has the same type of ovary as observed in monogamous seahorses. *Biology Letters* **4**: 362-365. Doi:10.1098/rsbl.2008.0157
- Stearns, S. 1992. *The evolution of life histories*. Oxford: Oxford University Press.
- Stearns, S. C. 1976. Life-history tactics - review of ideas. *Quarterly Review of Biology* **51**: 3-47.
- Stölting, K. N. & Wilson, A. B. 2007. Male pregnancy in seahorses and pipefish: beyond the mammalian model. *Bioessays* **29**: 884-896. Doi:10.1002/bies.20626
- Summers, K., McKeon, C. S. & Heying, H. 2006. The evolution of parental care and egg size: a comparative analysis in frogs. *Proceedings of the Royal Society of London Series B-Biological Sciences* **273**: 687-692. Doi:10.1098/rspb.2005.3368
- Svensson, I. 1988. Reproductive costs in two sex role reversed pipefish species (Syngnathidae). *Journal of Animal Ecology* **57**: 929-942.
- Takegaki, T. & Nakazono, A. 1999. Responses of the egg-tending gobiid fish *Valenciennes longipinnis* to the fluctuation of dissolved oxygen in the burrow. *Bulletin of Marine Science* **65**: 815-823.
- Takegaki, T. 2001. Environmental factors affecting the spawning burrow selection by the gobiid *Valenciennes longipinnis*. *Journal of Fish Biology* **58**: 222-229. Doi:10.1006/jfbi.2000.1438
- Takegaki, T. 2003. Factors affecting female parental investment in the monogamous goby, *Valenciennes longipinnis*. *Hydrobiologia* **510**: 147-152.
- Trivers, R. 1972. Parental investment and sexual selection. In *Sexual selection and the descent of man* (ed. B. Campell), pp. 136-179. Chicago: Aldine.
- van den Berghe, E. P. & Gross, M. R. 1989. Natural-selection resulting from female breeding competition in a Pacific salmon (coho, *Oncorhynchus kisutch*). *Evolution* **43**: 125-140.
- van Iersel, J. J. A. 1953. An analysis of the parental behaviour of the male three-spined stickleback (*Gasterosteus aculeatus* L.). *Behaviour (Suppl. 3)*: 1-59.
- Velez, M. J., Jennions, M. D. & Telford, S. R. 2002. The effect of an experimental brood reduction on male desertion in the Panamanian blue acara Cichlid *Aequidens coeruleopunctatus*. *Ethology* **108**: 331-340.
- Vigliola, L. & Meekan, M. G. 2002. Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. *Oecologia* **131**: 89-93.
- Vincent, A., Ahnesjö, I., Berglund, A. & Rosenqvist, G. 1992. Pipefishes and seahorses - are they all sex-role reversed? *Trends in Ecology & Evolution* **7**: 237-241.
- Vincent, A., Ahnesjö, I. & Berglund, A. 1994. Operational sex-ratios and behavioral sex-differences in a pipefish population. *Behavioral Ecology and Sociobiology* **34**: 435-442.
- Vincent, A. C. J., Berglund, A. & Ahnesjö, I. 1995. Reproductive ecology of five pipefish species in one eelgrass meadow. *Environmental Biology of Fishes* **44**: 347-361.
- Wallace, R. & Selman, K. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. *American Zoologist* **21**: 325-343.

- Wickett, W. P. 1954. The oxygen supply to salmon eggs in spawning beds. *Journal Fisheries Research Board of Canada* **11**: 933-953.
- Williams, G. 1966. *Adaptation and natural selection*. Princeton, N.J.: Princeton University Press.
- Wilson, A. B., Vincent, A., Ahnesjö, I. & Meyer, A. 2001. Male pregnancy in seahorses and pipefishes (family Syngnathidae): rapid diversification of paternal brood pouch morphology inferred from a molecular phylogeny. *Journal of Heredity* **92**: 159-166.
- Wilson, A. B., Ahnesjö, I., Vincent, A. C. J. & Meyer, A. 2003. The dynamics of male brooding, mating patterns, and sex roles in pipefishes and seahorses (family Syngnathidae). *Evolution* **57**: 1374-1386.
- Wisenden, B. D., Unruii, A., Morantes, A., Bury, S., Curry, B., Driscoll, R., Hussein, M. & Markegard, S. 2009. Functional constraints on nest characteristics of pebble mounds of breeding male hornyhead chub *Nocomis biguttatus*. *Journal of Fish Biology* **75**: 1577-1585. Doi:10.1111/j.1095-8649.2009.02384.x
- Wolf, J. B. & Wade, M. J. 2009. What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society B-Biological Sciences* **364**: 1107-1115. Doi:10.1098/rstb.2008.0238
- Wootton, R. J. 1990. *Ecology of teleost fishes*. London - New York: Chapman and Hall.
- Zahavi, A. 1975. Mate selection - a selection for a handicap. *Journal of Theoretical Biology* **53**: 205-213.