



GÖTEBORGS UNIVERSITET

# **Paternal care and brood reduction in a pipefish**

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### **Abstract**

From an evolutionary and adaptive perspective, the occurrence and extent of parental care in animals (investment in offspring beyond the genetic contribution) is expected to depend on an optimal (fitness-maximizing) balance of its costs and benefits. Males of the sex-role reversed broad-nosed pipefish, *Syngnathus typhle*, which care for eggs and embryos and may perform brood reduction, are particularly interesting in this respect. In this thesis, I explore paternal care in the broad-nosed pipefish with special focus on the causes and consequences of brood reduction.

Firstly, I demonstrate that brooding males absorb nutrients from reduced embryos in their brood pouch, presumably benefiting their own nutrition. This indicates that brood reduction in this species is similar to filial cannibalism (eating of own young), found in many other fish species with paternal care. Furthermore, as the embryos did not absorb nutrients originating from siblings in the same pouch, there is no support for 'nurse eggs' in this pipefish species (**paper I**).

When comparing brooding males with non-brooding males, brooding males were on average in better condition, as measured by hepatosomatic index (HSI). This is contrary to the expectation that the metabolic and other costs of brooding males should deplete their energy reserves. However, given the results above (**paper I**), the higher HSI in brooding males may to some extent stem from nutrients acquired through brood reduction (**paper V**). This uptake is, however, unlikely to fully compensate for the costs of paternal care since another experiment (**paper IV**) showed that males brooding full broods had lower survival, despite a larger relative brood reduction, compared to males with half-sized broods (**paper V**). Furthermore, the more eggs a male received, the more eggs were reduced, which supports the indication that the cost of brooding is related to brood size (**paper II** and **IV**). This clearly indicates that embryos are competing over paternal care and that paternal care is a costly and limiting resource.

Costs of paternal care in terms of reduced energy reserves (HSI) and lower survival (**paper IV**) suggests that males in poor condition (low HSI) should have a lower optimal brood size and thus show a larger relative brood reduction, as found in **paper III**. Also, males with low HSI received fewer eggs at mating and were more likely to suffer mortality from an infection (**paper III**).

When comparing monogynously and polygynously mated males, only monogynous males showed significant brood reduction, whereas polygynous males did not (**paper II**). This may be caused by either increased paternal provision or less competition due to less overlapping needs within half-sib than full-sib broods. Also, broods from larger females survived better than broods from smaller females (**paper III** and **V**). This may show that large eggs have certain qualities which allow them to survive better, or that males provide better care to eggs from larger females, reflecting the sexual conflict of embryo competition and cryptic male choice that may be operating in this species.

In summary, I have found that several factors influence the process of brood reduction (filial cannibalism) in the broad-nosed pipefish. These factors include the number of female mating partners, brood size, egg size and male condition. Consequently, these factors are important in parental conflicts and parent-offspring conflicts over the level of parental care and brood reduction.

**Keywords:** Parental care, brood reduction, embryo survival, parental condition, filial cannibalism, post-mating sexual selection, HSI, sexual conflicts, *Syngnathus typhle*, male brooding.

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## List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

- I. Sagebakken, G., Ahnesjö, I., Mobley, K.B., Braga Goncalves, I. & Kvarnemo, C. (2010) Brooding fathers, not siblings, take up nutrients from embryos. *Proceedings of the Royal Society B – Biological Sciences*. 277: 971-977.
- II. Sagebakken, G., Ahnesjö, I., Braga Goncalves, I. & Kvarnemo, C. (2011) Multiply mated males show higher embryo survival in a paternally caring fish. *Behavioral Ecology*. 22: 625-629.
- III. Sagebakken, G., Ahnesjö, I. & Kvarnemo, C. Male pipefish in good condition show less brood reduction. Submitted.
- IV. Sagebakken, G., Ahnesjö, I. & Kvarnemo, C. Male care on a budget: fitness consequences and brood reduction in relation to brood size in a pipefish. Manuscript.
- V. Sagebakken, G., Ahnesjö, I. Björnsson, B.Th. & Kvarnemo, C. Caring fathers and their energy reserves: a growth hormone experiment on pipefish. Manuscript.

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# Introduction

## Costs and benefits of parental care

Animals of most species provide no care to their offspring after fertilization. So why have some species evolved extensive parental care? The evolutionary answer is that parental care is expected to exist when the fitness benefits of care outweigh the costs of care (Clutton-Brock, 1991; Smiseth et al., 2012)

The benefits of parental care may be obvious; to increase the offsprings' survival and reproduction (Clutton-Brock, 1991). This can be done by protecting the offspring from predation and disease, to nourish or feed the offspring, or to otherwise enhance their quality or their surrounding environment (Sloman and Buckley, 2011). A result of parental care is often that the offspring are larger and more developed when the parental care phase is over, which may increase the survival and reproductive success of the offspring.

There are also several costs of providing care, potentially associated with parental survival and fecundity (Arnqvist and Rowe, 2005; Clutton-Brock, 1991; Maynard Smith, 1977; Sargent, 1997; Trivers, 1972, 1974; Westneat and Sargent, 1996). Investments in current progeny is ultimately at the expense of investments into future progeny, and natural selection can be expected to optimize the allocation of investment into the present relative to the future (Gross, 2005; Williams, 1966a). Hence, the amount of care provided by parents is to be optimized with regards to the costs and benefits of parental behaviours (Clutton-Brock, 1991).

## Conflict over parental care

Trivers (1972, 1974) used Hamilton's rule (Hamilton, 1964) to formulate a theory of conflict over the amount of parental investment that each offspring should receive from its parents. He found that offspring are typically selected to demand more parental investment than parents are selected to give (Trivers, 1972, 1974). The benefit for the offspring (and indirectly for the parent) would be enhanced survival and thus reproductive success, but with a cost in terms of future reproduction of the parent. There could also be a difference in the conflict level between full sibs and half sibs, since the theory of kin selection predicts that individuals should consider their inclusive fitness and adjust their behaviour to increase not only their direct fitness via their own reproduction, but also the indirect fitness of their relatives. Therefore, the interests of relatives often overlap, but less so for less closely related individuals (Griffin and West, 2002; Hamilton, 1964; Queller, 1992). Hence, by following Hamilton's rule one expects full sibs to cooperate to a greater extent than half sibs (Hamilton, 1964; Sachs et al., 2004). The difference in conflict level and cooperation between full siblings and half siblings may affect offspring survival when parental care resources are limited as siblings within the same brood will compete to monopolize resources for growth and survival (Lessells, 2002; Lessells and Parker, 1999; Magrath, 1990; O'Connor, 1978). If the embryos are not full sibling they may be less willing to share the resources and competition may be more pronounced (Lazarus and Inglis, 1986; Parker et al.,

2002; Trivers, 1974). Moreover, when egg size varies within broods, the larger embryos may have an advantage over smaller embryos through an increased tolerance to stress, an increased growth rate, or by otherwise being better in competing for resources (Marshall et al., 2008; Stearns, 1992). Also, bigger offspring may be of greater value to the parent and thus they provide more time or energy to ensure the “better” offspring’s survival (Brockelman, 1975; Clutton-Brock, 1991; Nussbaum and Schultz, 1989; Sinervo and McEdward, 1988).

### **Brood reduction**

Because of the often inevitable trade-off between offspring quantity and quality, broods may be adaptively reduced to ensure optimal size where the expected fitness return from the entire brood is maximised (Lack, 1947, 1948, 1954; Marconato et al., 1993; Williams, 1966b). Instead of all offspring receiving less care than they require and none of them surviving or succeeding to reproduce, the parent may have to choose which offspring to terminate care for. The parent may select between embryos based on their phenotypic quality or their genetic qualities. Likewise, parents are expected to adjust their parental care investment in relation to variation in the benefit to cost ratio from different types of offspring in such a way that it maximises parental fitness (Lessells, 2002). By terminating care for some embryos in the brood, the fitness of surviving offspring (Bonabeau et al., 1998; Brockelman, 1975) or the parents own future reproductive success may be enhanced (Clutton-Brock, 1991). However, the reduced young should only benefit from its own termination when the indirect fitness gain through siblings is greater than the sum of its own direct fitness in addition to the indirect fitness through siblings if it survives (Bonabeau et al., 1998; Brockelman, 1975; Hamilton, 1964). Brood reduction is most likely to be favoured early in the breeding attempt, since its potential benefits are higher and the costs are lower for both the parents and the siblings (Clutton-Brock, 1991).

Since Lack (1947, 1948) described brood reduction as the decrease in offspring number during the post-hatching period in birds, there has been a discussion if this is a mechanism parents utilize to adjust brood size adaptively (Mock, 1994; Ricklefs, 1965). In the broad sense, brood reduction is simply a decrease of offspring number while they are cared for. Mock (1994) defined the “narrow sense brood reduction” as the mechanism of brood reduction when offspring or the parents actively reduce the number of offspring in a brood to get higher fitness output. Narrow sense brood reduction by siblings has, for instance, been demonstrated in the great egret (Mock, 1987) and by the parent in the Cortez damselfish (Hoelzer, 1988). Sibling competition may also entail sibling cannibalism both before and after birth or hatching and exists in several taxa: birds (Stanback and Koenig, 1992), fish (Gilmore, 1993; Sargent, 1992; Wourms, 1977), starfish (Byrne, 1996), gastropods (Cubillos et al., 2007) and insects (Boots, 2000; Kukuk, 1992; Stevens, 1992). Some sharks practise intrauterine oophagy, which is when one of the offspring eats both fertilised and unfertilised eggs made available to them. The eggs consumed are called nurse eggs (Gilmore, 1993; Wourms, 1977, 1981).



Sibling competition resulting in brood reduction may reduce parental fitness (Parker et al., 2002; Trivers, 1974). However, when parents have more offspring than they are able to care for, brood reduction by sibling competition may enhance parental fitness (Mock and Forbes, 1995). A parent may also track the available resources in the environment, resulting in brood reduction under less favourable conditions. Furthermore, parents may initially accept more eggs than they can care for to ensure themselves against infertile eggs (Mock and Forbes, 1995; Mock and Parker, 1998). To enhance the quality of the remaining brood, caring parents may identify which offspring have the best fitness prospects out of the over-supply of offspring, and eliminate the others. This can be done by filial infanticide, by promoting siblicide actively, or by passively allowing offspring to be eliminated by fatal sibling competition (Forbes and Mock, 1998).

Filial cannibalism is another way to reduce the brood and is usually performed by caring males, probably since the benefit to cost-ratio of filial cannibalism is greater for the male than the female, as females are providing the eggs that are cannibalised. Filial cannibalism during care is more common in fish than in other organisms (Fitzgerald, 1992; Smith and Reay, 1991), and thought to be adaptive by increasing lifetime reproductive success of the parent (Rohwer, 1978). Since caring fathers may often lose weight and energy reserves, eating some of its own offspring may reduce such loss or even cause an increase in weight and energy reserves (Marconato et al., 1993), and increase the chance that the parent will reproduce again (Rohwer, 1978; Sargent, 1992). Filial cannibalism may also enable the male to better provide care for the remaining offspring and thereby increase their fitness, since males in good condition may be able to make a greater reproductive effort at lower immediate cost (Clutton-Brock, 1991; Trivers and Willard, 1973). Thus, if parental care has a survival cost, there will be an inverse relationship between adult and offspring survival, which has been found in some species (Klug and Bonsall, 2007; Kvarnemo et al., 1998; Manica, 2004) but not in others (Fitzgerald, 1992; Kraak, 1996; Rohwer, 1978).

### **Parental care in fish**

The free swimming larval phase in fish is generally considered more risk prone compared to the protected embryo stage, due to the fact that many predators feed on fish larvae (Wootton, 1998). Therefore, one can expect natural selection for an increase in parental care, if this results in more developed offspring with improved survival when reaching the risk prone larval phase (Shine, 1978; Sloman and Buckley, 2011). In fish, parental care can be performed in a number of ways, such as internal brooding, fanning or building a nest. These actions may improve the environment around developing embryos which may lead to higher developmental rates and higher embryo survival (Sargent, 1997). Some fish even provide mucus from their skin (Chong et al., 2005) or their mouth (Kishida and Specker, 2000) as a food source that enhances the survival and growth rate of the offspring. Furthermore, internal brooding protects the developing offspring from predators and diseases (Thibault and Schultz, 1978; Wourms, 1981). However, internal brooding often requires special adaptations to supply the embryos with oxygen to support the embryonic development

(Clutton-Brock, 1991; Keenleyside, 1979; Sargent, 1997). The caring parent may also provide the embryos with energy and nutrients. This may occur in various manners by using the other embryos as a food source (oophagy and adelphophagy), by transfer of nutrient from the parent to the offspring (through placental analogues)(viviparity), or through a yolk sac placenta (ovoviviparity) (Wourms, 1981; Wourms et al., 1988).

In viviparous species there may be parent-offspring conflicts as parents supply the limited resources (e.g. nutrients and oxygen), and sibling competition as the offspring may have to share these resources between themselves within or between broods. Also, since an offspring is more related to itself than to the parents, offspring are selected to acquire more parental resources than the parents allocate to each of them. Hence, the optimal brood size for parents is bigger than for the offspring in the brood (Mock and Parker, 1997). Furthermore, the two parents may differ in what the optimal brood size is, and the outcome of this conflict depends on the benefit and cost to each of the parents and which of them is in control of the brooding situation (Arnqvist and Rowe, 2005; Lessells, 2012; Lessells and Parker, 2012).

### **Study species**

The broad-nosed pipefish (*Syngnathus typhle*) lives along the coast of Europe in meadows of eelgrass (*Zostera marina*). It mostly swims vertically and also rests in the eelgrass. The broad-nosed pipefish have the same shape and range of colour as the eelgrass, which makes them very cryptic. The pipefish have a lengthy and ritualized mutual courtship dance before they copulate (Berglund and Rosenqvist, 2003). During copulation the female uses her “ovipositor” to insert eggs into the male brood pouch. While eggs are transferred to the male the pair rises in the water column until copulation ends. Thereafter the male sinks to the bottom assuming a S-shaped posture (Fiedler, 1954). Although it is not known in any detail when the eggs are fertilised, it has been suggested that it occurs while the male is in this S-posture. A male may brood eggs from several females in his brood pouch, and a female may deposit her eggs in several males’ pouches (Jones et al., 1999). Reproductive success positively correlates with body size in both males and females, as larger females produce an increased number and size of eggs (Braga Goncalves et al., 2011), and larger males can fit larger broods into their brood pouches (Ahnesjö, 1996; Berglund et al., 1986b, a, 1988)(figure 1a). In Syngnathids the male also has a 100% paternity assurance (Jones and Avise, 1997, 2001; Jones et al., 1999). The male brooding period ends with independent young leaving the brood pouch.

Generally, brood reduction is common in pipefish, and particularly in the broad-nosed pipefish (Ahnesjö, 1992a, 1996; Partridge et al., 2009; Ripley and Foran, 2006)(figure 2a). It has been shown that small eggs in broods of mixed egg size have a lower survival rate than both large eggs in such mixed broods and small eggs brooded alone (Ahnesjö, 1996 ). Based on these results, it was suggested that large eggs may be competitively superior to smaller eggs, that paternal provision may differ depending on egg size, or that pipefish use nurse eggs, or through an indirect route via the male’s blood stream to support embryo

growth of the other young (Ahnesjö, 1996; Ripley and Foran, 2006; Watanabe et al., 1999). It is important to point out that this does not necessarily imply brood reduction in the narrow sense, as they may only utilise energy and nutrients made available in the brood pouch from embryos that did not develop or developed too slowly (Cubillos et al., 2007; Forbes and Lamey, 1996). However, mechanisms for narrow sense brood reduction may work through filial cannibalism or through embryos competing for paternal resources, such as nutrition and oxygen.

### **The brood pouch**

The Syngnathid family (pipefish and seahorses) contains both ovoviviparous and viviparous species (Herald, 1959; Ripley and Foran, 2006; Stölting and Wilson, 2007). The species with more enclosed brood pouches generally have greater anatomical complexity and secretory function (Carcupino et al., 2002; Monteiro et al., 2005). Species with less complex brood pouches are thought to spend less energy brooding embryos than do males with more enclosed pouches (Herald, 1959). However, this may vary between species with the same pouch complexity (Ripley and Foran, 2006; Stölting and Wilson, 2007; Wilson et al., 2003; Wilson et al., 2001; Vincent and Giles, 2003).

To have a functional placenta-like structure there must be several adaptations concerning gas exchange, transfer of nutrients, disposal of waste products and suppression of immunological interactions between the parent and embryos (Haig, 1993). In an enclosed Syngnathid brood pouch the blood vessels together with the pouch matrix that fills the pouch, wrap the embryos and fill the entire space between them (Drozdov et al., 1997)(figure 2b). In at least three species of syngnathids, *Syngnathus fuscus*, *S.floridae* (Ripley and Foran, 2009) and *S. typhle* (Kvarnemo et al., 2011) there is evidence for nutrients being transferred from the brooding male to the developing embryos. This might happen via capillaries in the epithelium of the pouch walls that may transport nutrients from the paternal blood to the pouch by transcytosis, or the nutrients may be synthesized or modified in the epithelial cells (Ripley and Foran, 2006). The more complex pouches may also have mitochondria rich cells present (Carcupino et al., 1997, 2002). Furthermore, steroids and growth hormone may be transferred to the embryos, but this function has yet to be fully investigated (Azzarello, 1991; Haresign and Shumway, 1981; Ripley and Foran, 2006). Paternal care presumably has costs, as illustrated by the metabolic demands of the dwarf seahorse male, *Hippocampus zosterae*, which increase during gestation between 10% and 52% above the pre-mating level (Masonjones, 2001).



Figure 1. The broad-nosed pipefish male a) with a full brood pouch. b) with a less than half full brood pouch.

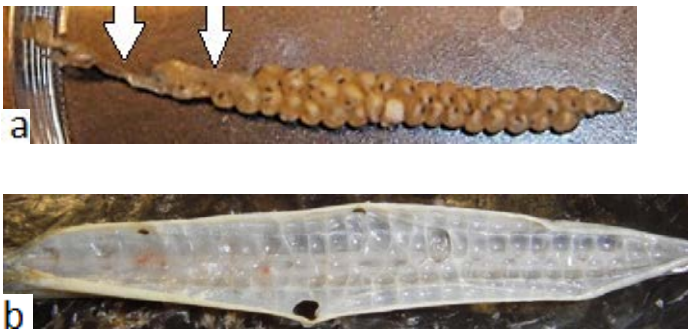


Figure 2. a) Embryos with spaces where brood reduction has occurred (arrows). b) The brood pouch emptied of embryos. The space where each embryo has been lying is clearly visible.

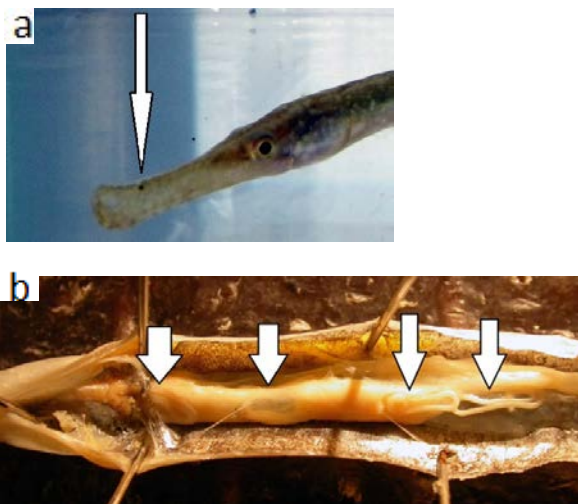


Figure 3.  
 a) The head of a male broad-nosed pipefish showing one *Cryptocotyle sp.* (arrow).  
 b) The intestines of a male broad-nosed pipefish at dissection showing 4 nematodes (arrows).

## Aims of the thesis

The overall aim for this thesis was to investigate what factors affect brood reduction in the broad-nosed pipefish (*Syngnathus typhle*, figure 1). I started with investigating where the nutrients from disappearing embryos end up (**paper I**), and considered the possibility of filial absorption and possible reallocation to the other siblings, or nurse eggs for siblings. In **Paper II** my aim was to investigate the effect of number of female mating partners (i.e. single or double maternity) on the occurrence and level of brood reduction. In **paper III** I investigated if brood reduction correlated with paternal condition and egg size. In **paper IV** I investigated if the initial number of eggs affected the level of brood reduction and the male condition. In **paper V** I investigated costs of paternal brood care, by manipulating males with growth hormone to see how brooding and growth hormone influenced brood reduction, condition and activity in males.

## Methods

Collection of pipefish and the laboratory studies were conducted at the Sven Lovén Center for Marine Sciences, Kristineberg, which is situated at the mouth of the Gullmar fjord, on the Swedish west coast (58°15'N, 11°28'E). The research station provided experimental rooms for aquaria with running natural seawater, taken directly from the fjord, thermo-regulated experimental rooms and suitable laboratories.

Fish were captured using a small beam trawl pulled by a small motor boat. Fish were caught within two kilometres distance from the research station. In the laboratory the pipefish were sorted according to sex and size. All fish were kept in storage barrels (200-225 litres) with running natural seawater kept at 14-15 °C. The light regime was artificial, but set to approximately follow natural hours for the season (between 16 - 19 h of light). Fish were fed three times a day with cultivated *Artemia sp.* and freshly caught Copepods, *Crangon crangon*, *Praunus flexuosus* and *Neomysis integer*.

### Fish identification

To be able to identify individual males over an extended period of time, I tattooed or colour marked the males, or both, depending on how many males were kept together. The males were sedated in 2-phenoxyethanol (100 µl/l) and marked subcutaneously with non-toxic latex paint (Liquitex™, New Jersey) using a G30 syringe, and/or tattooed with a black dot using a carbon-based, non-toxic ink applied by a tattoo needle on either side of a male's tail.

## Condition

I used different condition proxy measurements depending whether or not the calculated condition was on live or dead fish. In all papers I used male body length (standard length) as an estimate of condition or mate quality, as there is a female preference for larger males (Berglund and Rosenqvist, 1993, 2003; Berglund et al., 1986a). Larger males are also thought to provide better parental care for the embryos possibly because they maintain higher oxygen levels in the pouch during brooding (Berglund et al., 1986a; Braga Goncalves, 2010).

A thick upper body (trunk) relative to body length can also indicate good condition, in terms of larger energy and nutrition reserves. I therefore calculated residuals of fish trunk width regressed on fish standard length, as an estimate of condition in **paper IV**.

Feeding studies on other species of fish have shown the liver to be highly responsive to food intake (Gaylord and Gatlin, 2000; Luo et al., 2009; Turano et al., 2007). The hepatosomatic index (HSI) is therefore a good indicator of the energetic status of the fish (Hussey et al., 2009; Wootton, 1998). Thus, liver mass compared to body mass is a third estimate of body condition. It can only be measured on dead fish, since the liver has to be removed and a dry weight for both the liver and the rest of the body has to be taken. Hence, HSI is calculated as  $[\text{liver dry weight}] \times 100 / [\text{body dry weight}]$ . HSI was used as an estimate of condition in **papers III, IV and V**.

I also did lipid extraction of the whole fish following the methods of Lissåker et al. (2003) on all the fish from 2008 to 2010. However, as the fat content in the fish is low with mean percentage ( $\pm$  S.E.) varying between years: 0.63 ( $\pm$  0.49) for 2008, 0.53 ( $\pm$  0.13) for 2009, and 0.82 ( $\pm$  0.35) for 2010, the fat reserves to deplete in this fish are very small. Also, there was very little variation in fat levels between fish. Furthermore, the fat percentage did not differ between euthanized and surviving males (t-test,  $t = 0.99$ ,  $df = 362$ ,  $P = 0.32$ ). Hence, fat is not a good measurement of condition in this species, and was not used in the analysis.

## Egg counting and brood reduction calculations

After mating, the males were sedated in 2-phenoxyethanol (100  $\mu$ l/l) and the initial number of eggs were counted using a cold light, shone through the pouch and counted up to three times. Counting eggs in live fish involves some degree of counting error, sometimes resulting in less than 0 % brood reduction. Importantly, the egg counting error should be the same between treatment groups and should therefore not bias the results. The males were then allowed to brood the embryos for a number of days, before being euthanized. The number of days varied slightly between studies (**paper I**: 16 days, **paper II**: 17 days, **paper III**: 14 days, **paper IV**: 26 days, **paper V**: 18 days). Because it takes around 35 days from mating till parturition in 14 – 15 °C water (Ahnesjö, 1995), these experimental brood periods correspond to 40 – 75 % of a whole brooding period. After the males had been euthanized the embryos were taken out of the brood pouch and counted once again. I calculated relative brood reduction as  $[\text{the number of normally developed embryos}] \times 100 / [\text{the number of eggs initially received}]$  following the methods of Ahnesjö (1992b, a, 1996).

### **Embryo weight**

After embryo counting I took 10 embryos from the lower part of the brood pouch and 10 from the upper part of the brood pouch (if applicable) and dried them for 36 hours at 70 °C. Then I weighed them on a Sartorius LE26P microbalance to the nearest 0.01 mg and calculated the average embryo weight separately for upper and lower pouch. After checking that there were no significant difference between the upper and lower pouch embryo weights, I pooled the two samples and calculated the average embryo weight per male.

### **Radioactivity**

In **paper I** a  $^{14}\text{C}$ -labelled amino acid mixture (Perkin Elmer™, [ $^{14}\text{C}(\text{U})$ ]-L-amino acid mixture, product number NEC445E050UC) was used to make eggs radioactive. Large and small females were given 1  $\mu\text{Ci}$  each of this substance directly into the stomach using a thin tube attached to a Hamilton syringe. Large and small females were also given water as control to ensure that all the females received the same handling. The mating treatments were as follows: Either males received roughly half their broods from large non-labelled females and half from small  $^{14}\text{C}$ -labelled females, or half from large  $^{14}\text{C}$ -labelled females and half from small non-labelled females. Consequently, approximately half of the embryos in the male's brood pouch were radioactive. Control males were mated to small and large non-labelled females. After mating, males were left to brood for up to 16 days before they were euthanized. Samples were taken from embryos and male pouch tissue above these embryos, liver and muscle tissue to trace the radioactivity that emanated from the  $^{14}\text{C}$ -labelled eggs.

Each tissue sample was dried and weighed to the nearest 0.01 mg. Then the tissues were dissolved with Soluene-350® (Perkin Elmer™) in a water bath set to 65°C for at least 21 hours. Scintillation fluid (Hionic-Fluor, Perkin Elmer™) was then added to each vial and the samples were left to develop in a dark cupboard for at least 24 hours, before being analysed in a liquid scintillator (Beckman Coulter LS 5000TD), counting disintegrations per minute (dpm). To correct for different kinds of tissue blocking the signal to different extents, I prepared separate quench curves for the different tissues. I used the  $^{14}\text{C}$  radioactivity in the control males' tissues to set the background level of radioactivity, and tested if the different tissues of experimental males showed an increase in radioactivity.

### **Growth hormone implants**

In **paper V**, I used a long-term manipulation of growth hormone (GH) levels by introducing sustained release bovine GH implants into the body cavity of the fish. Bovine GH is a heterologous mammalian hormone that has been used previously in studies on fish (Björnsson et al., 1994; Leedom et al., 2002; Peterson et al., 2004; Raven et al., 2012; Weatherley and Gill, 1987). I injected GH to manipulate the males to invest more energy into self (growth, metabolism, activity), and hence possibly less into current and/or future reproduction and survival.

## Parasites and diseases

I measured the frequency of the three most apparent parasite infections *Gyrodactylus sp.*, *Cryptocotyle sp.* (figure 3a), and nematodes (figure 3b) between 2007 and 2010, except in 2009 when I only recorded nematodes (table 1). In 2008 there was a virulent infection, most likely caused by *Vibrio sp.*, and in 2010 a fin rot or fungal infection. Most likely the *Vibrio sp.* infection caused 53 of 64 fish to die in 5 days in May 2008. The 2010 infections were treated with an anti-fungal bath. Yet, in only 6 days 85 fish out of 218 died as a result of the infection.

Table 1: The incidence (%) of infection among all fish that were investigated, and the magnitude of infection in infected fish (range and mean  $\pm$  S.E.) in the male broad-nosed pipefish carrying natural levels of *Gyrodactylus sp.*, *Cryptocotyle sp.* and internal nematodes for the years 2007 to 2010. N/A means the data are not available, - means not applicable.

YEAR	GYRODACTYLUS SP.	CRYPTOCOTYLE SP.	NEMATODE
<b>2007</b>	92 %	63 %	49 %
Range	1 - 34	1-7	1 - 9
N = 49	8.40 $\pm$ 1.25	2.17 $\pm$ 0.40	2.08 $\pm$ 0.37
<b>2008</b>	6 %	61 %	66 %
Range	1 - 3	1 - 59	1 - 12
N = 69	1.5 $\pm$ 0.5	4.83 $\pm$ 1.23	2.37 $\pm$ 0.29
<b>2009</b>	N/A	N/A	8 %
N = 74	N/A	N/A	1
<b>2010</b>	0 %	52 %	19 %
Range	-	1 - 47	1 - 8
N = 228	-	5.56 $\pm$ 0.65	1.67 $\pm$ 0.20

## Summary of papers

### Paper I: Embryo absorption by brooding fathers

Previous research on the broad-nosed pipefish have found brood reduction during paternal brooding (Ahnesjö, 1992a, 1996; Partridge et al., 2009). However, it was unknown where the nutrition from reduced embryos ends up. Thus, the purpose of the experiment in **Paper I** was to examine where such resources go, with the specific aim to test if there is an uptake of nutrients by brooding males or siblings. Males mated with both radioactive and non-radioactive females, thus half the brood in their pouch were radioactive. After about two weeks of brooding I euthanized the males and traced the radioactivity in different tissues



and embryos in order to determine if there was a) only a paternal uptake, b) only an uptake by unlabelled siblings in the pouch, or c) both paternal and sibling uptake.

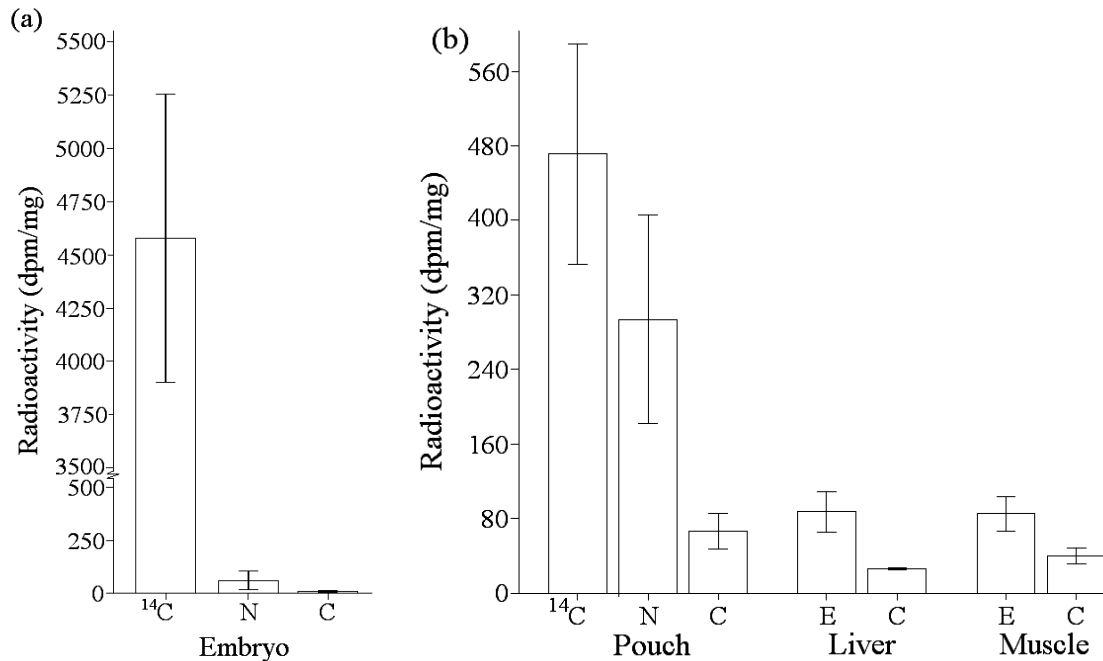


Figure 4. Radioactivity measured on *Syngnathus typhle* as disintegrations per minute per milligram tissue (dpm/mg, mean  $\pm$  S.E.). The samples were taken from (a)  $^{14}\text{C}$ -labelled embryos ( $^{14}\text{C}$ ), non-labelled embryos (N) in experimental males, and embryos from control males (C), and (b) different tissues from brooding males. In the experimental males tissue samples were taken from pouch above  $^{14}\text{C}$ -labelled embryos ( $^{14}\text{C}$ ), pouch above non-labelled embryos (N), and in control males pouch (C) was taken. Liver and muscle tissues were taken from experimental (E) and control (C) males. Note the break in the scale in panel (a). (Reprint from paper I).

The non-labelled embryos did not show any significant increase in the  $^{14}\text{C}$ -radioactivity level, compared to the embryos from control males (figure 4a). However, I found that brooding males take up radioactivity (i.e. nutrients from embryos in their brood pouch), as I found a significant increase in radioactivity in brood pouch tissue, liver tissue and muscle tissue (figure 4b). It is reasonable to assume that this radioactivity emanates from labelled embryos that have been reduced in the pouch, thus representing a paternal uptake of resources and nutrients.

Because of uptake of radioactivity from the embryos to the brooding male was observed, suggests that males can absorb nutrients from their broods, in a way that strongly resembles filial cannibalism, found in many other families of fish (Fitzgerald, 1992; Manica, 2002; Sargent, 1992). Also, I did not find any support that males reallocate the nutrition taken up to the other embryos, at least not within the first two weeks of brooding, or that the other embryo group can absorb nutrients directly from their half siblings. Hence, in this

species of pipefish the suggestion of supplementary feeding of embryos with nurse eggs (Ripley and Foran, 2006; Watanabe et al., 1999) or half-sibling cannibalism is not supported.

### **Paper II: Multiply mated males show higher embryo survival**

The broad-nosed pipefish population investigated here is one of the more polygynously mating populations in Europe (Rispoli and Wilson, 2008). In most other taxa, the benefit for males mating with several females is an increased number of eggs fertilized. However, in this species once the brood pouch is full, the male cannot increase the number of eggs fertilized by multiple mating, as the brood pouch has limited space. Still, there is variation in embryo survival, which affects the actual number of offspring released from the brood pouch.

In **paper II**, I experimentally investigated the effect of the number of female mating partners on brood reduction. Males were mated to either one or two females and thus comparisons could be made between broods of half or full siblings. If brood reduction was higher in the half sib broods, males might promote full sib broods over half sib broods, or there may be more intense sibling competition in half sib broods. The latter could also reflect female-female competition after mating in this species that is characterised by female-female competition before mating (Berglund and Rosenqvist, 2001; Eberhard, 1996). If brood reduction, on the other hand, is higher in the full sib broods, it can indicate that males promote half sib broods, or that full sibs are more prone to sacrifice themselves to increase the fitness of their siblings. Full sib broods may also have more overlapping care demands, which could lead to an increase in brood reduction in full sib broods. If brood reduction is unrelated to number of females that contribute to broods, it would indicate that brood reduction is due to fertilization failure or other factors unrelated to number of females contributing to a brood.

In this study I found that the singly mated males on average showed a significant amount of brood reduction, whereas the doubly mated males did not (figure 5). Males that received more eggs initially also showed higher relative brood reduction.

The higher survival of half sib broods might thus be a result of greater support given by the father to these broods. One example of why these broods may be of greater value to the caring father is their increased genetic diversity compared to full sib broods. Similar promotion of a genetically more diverse broods has been found, for example, in pygmy grasshoppers and fruit flies (Forsman et al., 2007; Jennions and Petrie, 2000; Taylor et al., 2008). If the pipefish habitat fluctuates or varies in such a way that the best genetic combination cannot be predicted, a brooding male may be better off producing more genetically diverse offspring (half siblings) than more genetically similar ones (full siblings) (Forsman et al., 2007; Yasui, 1998). The other explanation for increased survival in polygynous broods would be that half siblings may experience reduced competition in the brood pouch due to different genotypes utilising different resources at different times (Jennions and Petrie, 2000; Yasui, 1998). This means that full siblings would compete for limited resources more intensively than half siblings in the brood pouch, which may explain the higher level of brood

reduction in single mated males in this study. Kin selection can probably be ruled out as an explanation due to a lack of fitness gain by the other embryos, at least when it comes to increased body mass.

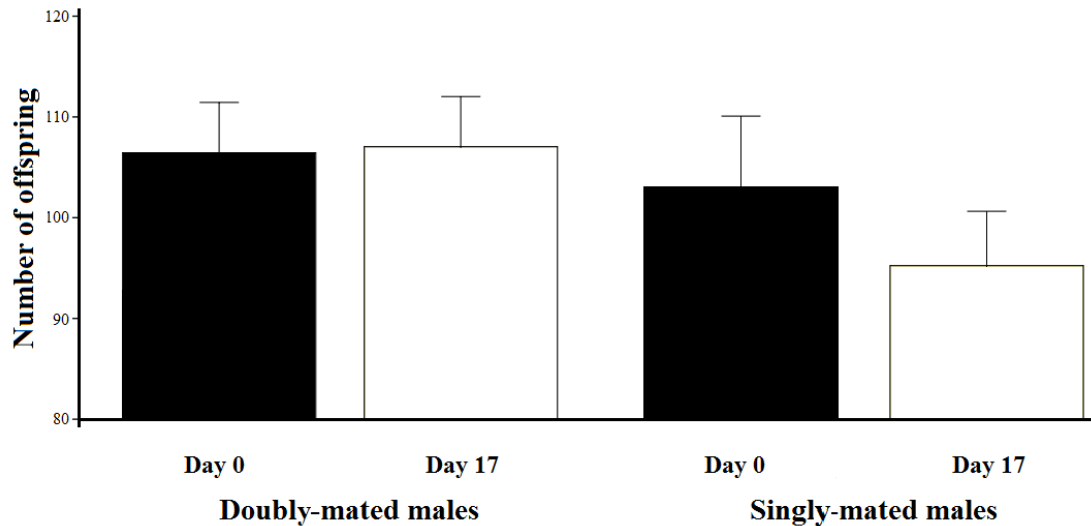


Figure 5: The mean number of eggs ( $\pm$  SE) received at mating (day 0) and the number of offspring supported after 17 days of brooding in males mated to two females (doubly-mated) or one female (singly-mated). Note: The y-axis starts at 80. (Reprint from paper II.)

The result that larger broods showed a higher degree of brood reduction may be explained by more embryos demanding more brood care in terms of factors such as oxygen or specific nutrients. Hence, brood reduction may thus be affected by the males' limited ability to care for a large number of embryos. This was further investigated in **papers III** and **IV**.

### **Paper III: Males with higher HSI show less brood reduction**

If parental care is a limiting resource, then within each male the parental condition and parental care should correlate negatively. In this paper I used HSI as a proxy for condition to investigate how male condition correlates with relative brood reduction.

First of all, and most importantly, I found that HSI correlated positively with the survival of brooding males. Furthermore, using a partial correlation (table 2), I confirmed a well-known relationship that longer males can brood a higher number of eggs, but length did not correlate with any of the other variables. Number of eggs and male HSI also correlated positively. This could either mean that males in better condition accepted or were offered more eggs from the females at mating, or that having a larger brood can improve male condition, possibly via the nutrient uptake demonstrated in **paper I**. This does not mean that parental care is cost free, but that males trade brood size against their own condition and survival. I will return to these issue in **papers IV** and **V**.

Table 2: Partial correlations (*i.e.* pairwise correlations while the other variables are kept constant) between male length, egg number at mating, hepatosomatic index (HSI), relative brood reduction and embryo weight in brooding broad-nosed pipefish males. N = 69. Significant correlations are marked in bold.

	Egg number at mating	HSI	Relative brood reduction	Embryo weight
Male length	<b>r = 0.79</b> <b>P &lt; 0.001</b>	r = -0.14 P = 0.28	r = -0.12 P = 0.33	r = -0.003 P = 0.98
Egg number at mating		<b>r = 0.25</b> <b>P = 0.04</b>	r = -0.01 P = 0.94	r = 0.03 P = 0.81
HSI			<b>r = -0.27</b> <b>P = 0.03</b>	r = -0.09 P = 0.48
Relative brood reduction				<b>r = -0.42</b> <b>P &lt; 0.001</b>

Males brooding heavier embryos had less relative brood reduction. Since larger young hatching from larger eggs are more likely to survive, males may be willing to provide more care which results in less brood reduction. However, larger eggs and embryos may also have some intrinsic quality that makes them survive better in the brood pouch.

Males with higher HSI had significantly less relative brood reduction, or *vice versa*. Hence, this result indicates that increased relative brood reduction in poor condition males may be because they cannot provide the amount of care needed to all the offspring.

Parental care may further reduce the condition of caring males. However, because embryo absorption provides an avenue for males to minimize any decrease in their own condition or the remaining offspring's condition, a brooding male can possibly choose not only between caring and not caring, but also between caring and consuming. This makes the relationship between parental care, parental condition and brood reduction increasingly complex. Females should prefer to mate with males in better condition since condition correlates positively with care. However, why females prefer to mate with large males, as found previously (Berglund et al., 1986a), is not clear from this data set. Males should also accept more eggs if they are in better condition, since this may make them more capable of caring for more eggs. However, males should also accept more eggs even if they are not able to care for them, if they can absorb the embryos and use them as an extra nutrient source.

#### **Paper IV: Fitness consequences and brood reduction in relation to brood size**

In this study, I investigated the effect of brood size on relative brood reduction and male condition, such as survival and hepatosomatic index (HSI). Brood size is an important fitness component, since larger broods have the potential to result in more offspring produced (Godfray et al., 1991). However, there may be trade-offs between brood size and other fitness components such as the parent's future reproductive success (Partridge and Harvey,

1988; Partridge and Sibly, 1991; Stearns, 1992; Williams, 1966b). Large broods may increase the mortality risk of offspring in the brood, and reduce or delay the parent's chance of breeding again. Moreover, just like the brood size is likely to be positively influenced by the caring parent's condition, the parental condition may be negatively affected by the number of offspring under care (Clutton-Brock, 1991; Sabat, 1994).

In this study each male was allowed to mate with a single female and to either fill up the brood pouch completely with eggs or to fill approximately half of the brood pouch. If embryo survival is linked to number of embryos in the brood, as indicated by the results in **paper II**, I would expect a full brood to show relatively higher levels of brood reduction. However, if embryo survival is unaffected by brood size I would expect no difference in relative brood reduction between the half and fully filled brood pouch treatments. If paternal resources are limited, I would also predict males in lower condition to show relatively more brood reduction, in accordance with the energy-based hypothesis (Rohwer, 1978; Sargent, 1992).

I found that males with a full brood pouch showed higher relative brood reduction, and poorer adult survival, compared to males brooding only half a brood (figure 6). This difference in relative brood reduction did not coincide with a difference in male condition (HSI) between the treatments, nor in number of days the broods had been brooded or in embryo weight. However, males from both treatments that were euthanized due to poor health had lower condition in terms of HSI and higher relative brood reduction. This is consistent with the energy-based hypothesis, since the quality of parental care depends on parental condition (Rohwer, 1978; Sargent, 1992). The higher relative brood reduction by these males indicates that males in very poor condition are less able to provide care. This result is also consistent with the negative correlation between male HSI and relative brood reduction found in **paper III**.

The number of offspring may be a key factor for the occurrence, and adaptation, of brood reduction in pipefish. Since broad-nosed pipefish males provide both nutrients and oxygen to the developing offspring (Braga Goncalves, 2010; Kvarnemo et al., 2011), increased survival of embryos in smaller broods can be explained by fewer offspring having to share the resources supplied to the brood by the male. However, it is clear that a parent with a small or reduced brood does not spend all of its saved resources on providing increased care per remaining offspring, but instead invests some in its own future reproductive success, as shown by the higher survival among males brooding small broods.

Since brood reduction was greater in larger broods, HSI might be expected to be higher in males brooding larger broods. However, HSI did not differ between treatments. Also, if care given by the males is constant, I would expect embryos of smaller broods to be heavier than embryos of larger broods. Interestingly, I found no difference in embryo weight between half and full broods. The results from the HSI and embryo weight taken together, may show that whatever a male may gain from reducing the number of offspring he cares for, may influence embryo weight in the remaining embryos, as has been suggested earlier (Ahnesjö, 1992a; Berglund et al., 1986a). This argument suggests that males are limited by

resources available for reproduction, and might trade number of new-born for their weight. Such a trade-off is likely to be adaptive, because survival is positively related to weight of the newborn (Ahnesjö, 1992b).

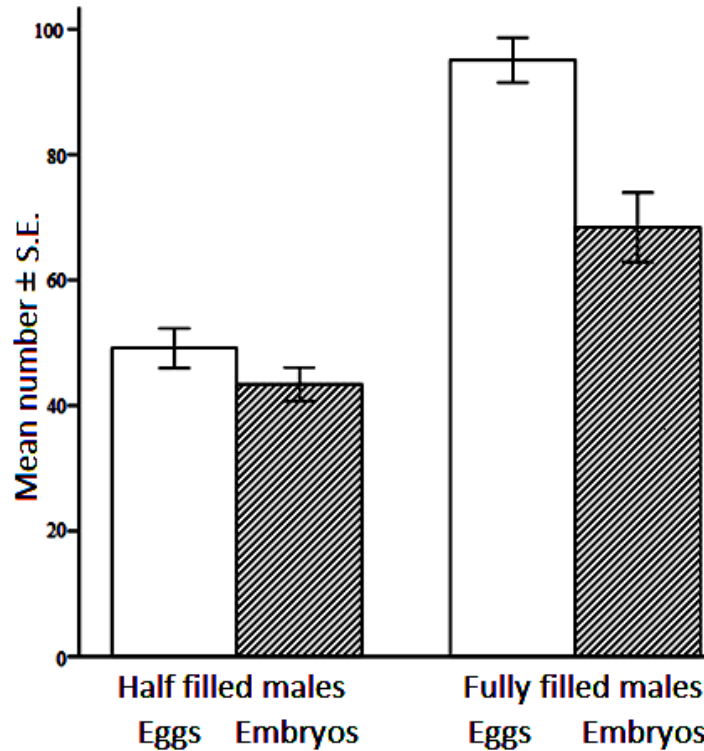


Figure 6. Mean number ( $\pm$  S.E.) of eggs at the time of mating (open bars), and embryos after 2/3 of the brooding period (hatched bars) in *S. typhle* males that were initially half filled or fully filled.

Density-dependent embryo survival has been linked to oxygen depletion in other species of fish (Lissåker et al., 2003; Payne et al., 2002), but see (Klug et al., 2006; Watanabe and Watanabe, 2002). By varying the number of eggs in the brood pouch in this study, the density of the embryos may also vary. However, half a brood does not mean that each embryo gets twice the space, because males fill their pouches with eggs from the bottom up, shaking them down after egg transfer. They also seal off empty upper parts of the pouch during brooding, if not fully filled (figure 1b). Therefore, although I cannot completely exclude that a reduced embryo density contributed to the higher relative survival of the young brooded in half-filled pouches, embryo density varied considerably less than embryo number.

#### **Paper V: Caring fathers and their energy reserves: a growth hormone experiment**

In this study broad-nosed pipefish males were injected with growth hormone (GH) to increase their activity and metabolic rate. I tested if an increased investment into ventilation and activity influenced brood reduction or male condition measured as survival and HSI.

Although parental care, by definition, increases offspring fitness, it also reduces the caring parent's future reproductive success, via a loss of body weight, energy reserves or survival. Hence, I tested the cost of brooding *per se*, by comparing the same parameters in brooding males versus non-brooding males.

I found that GH increased activity in both brooding and non-brooding males. However, only brooding males increased their ventilation rate when subjected to GH. GH did not increase male growth rate, feeding rate or relative brood reduction.

Brooding males had on average higher HSI than non-brooding males (figure 7). This is contrary to expectations since brooding males probably provide costly care to their developing embryos. However, the higher condition in brooding males may stem from brood reduction, since brooding males absorb nutrients from embryos through their brood pouch as found in **paper I**. Or the higher HSI in brooding males is an effect of, for instance, a more efficient food conversion. From previous **papers III and IV** I also got some indications that higher relative brood reduction may positively affect male energy reserves (HSI).

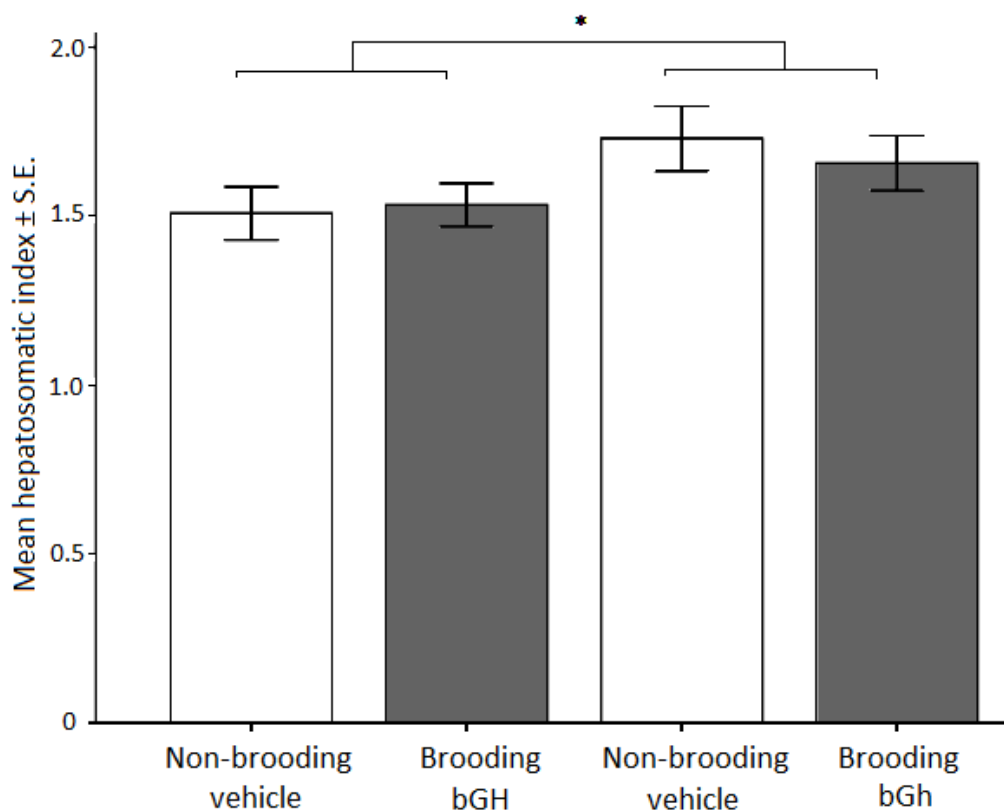


Figure 7. Hepatosomatic index (HSI) for the four different male groups. Gray bars represent GH-treated groups and open bars represent vehicle-treated groups. Brooding males have significantly higher HSI than non-brooding males (\*). Data are presented as means  $\pm$  S.E.

Males that brooded larger embryos, showed less relative brood reduction and were in better condition. Because large embryos originate from large females, this result might be an effect of larger embryos surviving better in the brood pouch since they are more successful in resource competition in the brood pouch. However, males may provide better care to eggs from larger females, possibly as a result of heavier embryos being more valued due to better fitness prospects (Ahnesjö, 1992a). The result that larger embryos were brooded by males with a higher HSI therefore suggests that large, competitively superior females choose males based on their condition, since it correlates positively both with male and offspring survival, as found in **papers III and IV**. Alternatively, eminent care by high condition males may have caused the embryos to become heavier. However, this is slightly less likely, because embryo size was unaffected by brood size in **paper IV**, but can of course not be excluded at this stage.

## Conclusions and Perspectives

In **paper I** I found that brood reduction in this pipefish species as a process can be viewed as an equivalent to filial cannibalism, that is expressed in other species of fish (Sargent, 1992), birds (Stanback and Koenig, 1992), rodents (Elwood, 1992), non-human primates (Hiraiwa-Hasegawa, 1992) and insects (Stevens, 1992; Thomas and Manica, 2003) among others. Brood reduction in pipefish is a special case where the placenta-like structure not only provides care to the offspring, but can be used to absorb resources from the brood. Brood reduction was found to be affected by several factors. Larger broods showed a higher degree of brood reduction, both in **Paper IV** where I experimentally manipulated initial brood size, and in **Paper II** where I found a positive correlation between relative brood reduction and brood size. This correlation may be caused by limited resources for paternal care. Broad-nosed pipefish males may thus be a parentally optimistic fish showing an adaptive brood reduction when resources are scarce (Mock and Forbes, 1992). Even though males are able to absorb reduced embryos (**paper I**), this can only partially compensate for the costs of brood care, clearly indicated by higher survival in males with experimentally reduced (halved) brood sizes (**paper IV**).

The cost of brood reduction is that fewer offspring are produced. One benefit of brood reduction is a reduced expenditure on care for a smaller brood, which may increase the male's chance of surviving the brooding period (**paper IV**). A full brood may benefit relatively more from brood reduction if embryo survival is related to number of offspring in the brood pouch (**paper II, III and IV**), as fewer offspring have to compete over paternal care resources.

In figure 9, I show three simplified scenarios of how an increased relative brood size may influence male condition after brooding. Illustrated by the lower line, in all three cases I assume that male condition decreases with increased brood size, if the possibility of embryo absorption via brood reduction is disregarded. This assumption is an extrapolation from **paper IV**, in which males with full broods had poorer survival than males with half broods.



Males in **paper IV** were not prevented from brood reduction, but because their brood sizes were manipulated, the scope for brood reduction was reduced for half filled males. The upper line shows expected male condition when embryo absorption through brood reduction is taken into account. Depending on whether males take up (a) a constant amount of resources regardless of brood size, or (b-c) an increasing amount with larger brood size, male condition can be expected to (a) decrease, (b) be unaffected or (c) increase with increased brood size. My result from **paper IV** shows that (b-c) are more likely than (a), because males with full broods showed higher brood reduction than males with half broods. But because condition when measured as HSI after the experiment in **paper IV** did not differ between males with half and full broods, it is possible that the scenario shown in (b) best reflects what happens in a real male. Although I cannot exclude that (c) might had become more accurate, if the brooding period had been longer, the fact that males in good condition after brooding showed less brood reduction (**papers III and IV**) indicates that there is a feedback that prevents males from excessive brood reduction. Thus, paternal condition probably affects the amount of care a male can give, as males in good condition should have more resources to spend on care, without having to resort to brood reduction.

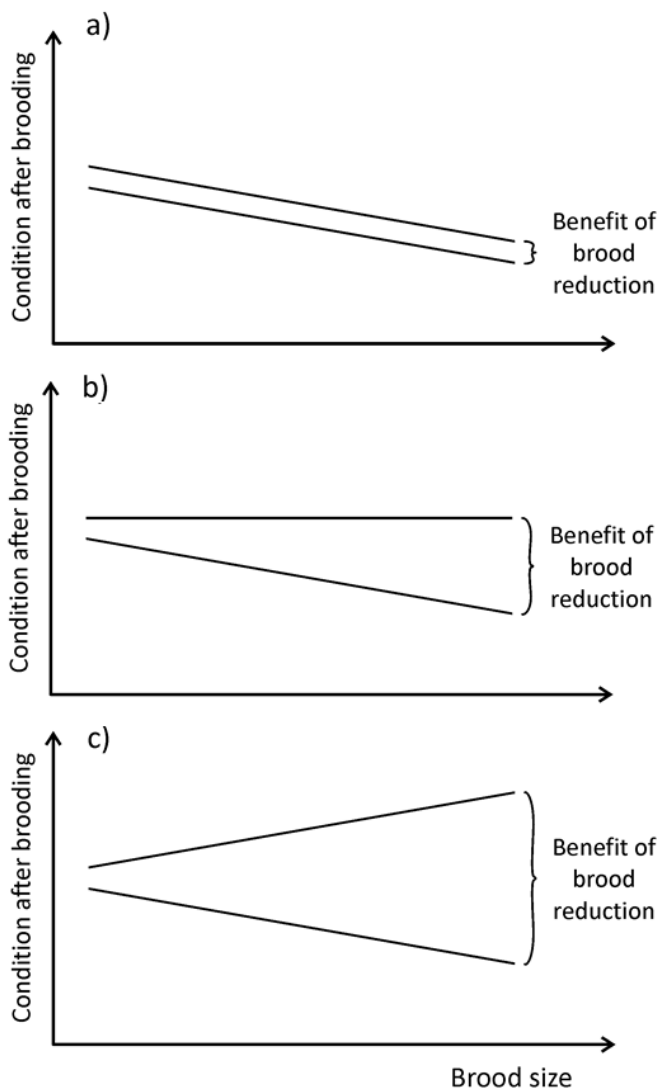


Figure 9. How initial relative brood size might affect male condition after a period of brooding. The lower line hypothetically shows how the male's own resources probably decline as the number of offspring under care rises. The upper line represents how the male's condition might be affected by the brood size when he also can take up nutrients from the offspring and use the extra acquired resources (a) to only slightly improve his condition and regardless of brood size, or to (b) stabilise or (c) enhance his condition with increased brood size. The gap between the upper and lower lines thus represents what the male gains in terms of condition from brood reduction.

### **Liver size, male condition and paternal care**

Caring males in other species of fish usually decrease liver weight when they enter the paternal care phase, as found in tilapia (Weber and Grau, 1999) and stream goby (Ito et al., 2010). However, the opposite was found in the broad-nosed pipefish (**paper V**). There may be many possible causes for the increased HSI of brooding males, but so far no such mechanism has been found. Brooding males might have increased food conversion efficiency, or any other physiological process, that will increase the liver size when males go in to the paternal care phase of reproduction. One mechanism that is known to cause an increased HSI is estradiol-17 $\beta$  (E2), since E2 increases vitellogenin production in the liver (Reading and Sullivan, 2011). In *S. scovelli* adding E2 to the fish' water caused the male liver size to increase to unusually large size above both female and male normal sizes (Ueda et al., 2005). However, because E2 normally decrease when males mate and start brooding in the broad-nosed pipefish (Mayer et al., 1993), this is not a likely alternative explanation to why HSI values increased in brooding males in **paper V**. Rather, the explanation that some of the nutrients from reduced embryos are stored in the male's liver (**paper I**) and result in increased liver weight in brooding males (**paper V**) seems to be the simplest explanation.

In this paternally caring species, the male option is not only to care or not to care, but also to care or to consume. However, males with low energy reserves may provide less paternal care in the form of nutrients and/or oxygen. Less paternal care may cause the embryos to compete harder over the care resources, and hence less fit embryos may die. At that point, the male may absorb nutrients emanating from these embryos. Also, it is possible that a higher brood reduction in the present brood may cause a lower brood reduction in the next brood and *vice versa* as has been speculated for *S. scovelli* (Paczolt and Jones, 2010).

The correlative evidence shown in **Paper III** that paternal condition affects brood reduction has implications for mate choice and conflicts between the parents. If males are in poor condition, they may adjust their paternal care to reduce the costs. Furthermore, I have evidence that brood reduction may influence male fitness positively by increasing the HSI (**paper V**). Also, one also has to take into account that the embryos a male receive may have different energetic demands, and that male quality may determine how much he can and will invest in his brood. Hence, males appear to accept more eggs when they are in good condition (**paper III**).

In general, females are expected to prefer caring males that will invest heavily into her offspring (Lindström, 1998; Lindström and St.Mary, 2008; Petrie, 1983). Hence, females should also be expected to offer more eggs to males in good condition, since the males' HSI correlates with their ability to care, as found in **paper III, IV and V**. I also found that brooding males with higher HSI have heavier embryos in their pouch, and also show less brood reduction. Because large embryos almost certainly come from large females (Braga Goncalves et al., 2011), which have a competitive edge in female-female competition for mates (Berglund and Rosenqvist, 2003), indicates that females prefer or at times even compete over males in better condition. Also, females prefer large male partners, and it has

been suggested that they do this because such males are able to invest more energy per offspring (Berglund et al., 1986a; Quast and Howe, 1980). Hence, large males will produce larger offspring with higher chance of survival than offspring from smaller males (Ahnesjö, 1992a; Dzyuba et al., 2006). In another study on the closely related species *S. schlegeli*, male body size was also negatively correlated with embryo density in the pouch, which in turn was negatively correlated with newborn dry weight (Watanabe and Watanabe, 2002). The growth rate of newborn pipefish was negatively correlated with the number of newborn emanating from a male brood pouch in *S. typhle* as well as in a seahorse species (Ahnesjö, 1992a; Dzyuba et al., 2006). Thus, it can be speculated that *S. typhle* males reduce their broods in order to optimize offspring birth weight, rather than maximize it, as discussed in **paper IV**.

### **Sexual conflicts over brood reduction**

A sexual conflict over the level of paternal care is likely to arise, as the optimal level of brood reduction or filial cannibalism *versus* care may differ for males and females (Lindström, 2000; Wedell et al., 2006). In addition, males have the option to parasitize on the female egg investment through embryo absorption (**paper I**). Full brood reduction disrupts the parental investment in the present brood and males may instead invest in their own growth and future reproduction. This is well known from fish and insects that show filial cannibalism (Manica, 2002, 2004; Stevens, 1992; Thomas and Manica, 2003). In contrast, partial brood reduction is not only investment into future reproduction, but may also be an investment into the current brood, since it may enable the paternal male to survive until the end of the brood cycle and ensure survival for the rest of the brood (Rohwer, 1978). Partial brood reduction may be a way to avoid spending care on embryos with no future prospects and at the same time take advantage of the resources in those embryos. Brood reduction may be a conflict area between the males and females in this fish. However, there might be less of a conflict over brood reduction if the male invests the energy gained to increase the quality of care for the rest of the brood (Lindström, 2000). However, brood reduction may be beneficial to both sexes, if the male invests the gained energy into providing better care for the rest of their joint offspring. However, if not, there will be a conflict between the sexes over brood reduction.

In animal behaviour research, the main focus in sexual selection has been on pre-mating selection in species with conventional sex-roles (Andersson, 1994; Arnqvist and Rowe, 2005; 1948; Shuster and Wade, 2003; Trivers, 1972). Sexual selection can also occur after copulation, as cryptic mate choice and gamete competition (Andersson and Simmons, 2006; Birkhead, 1998; Eberhard, 1996; 1970; Simmons, 2001, 2005). Post mating sexual selection in this sex-role reversed species would, if anything, entail female-female embryo competition and cryptic male choice. Females may compete between each other through their eggs in the brood pouch. Thus, half sib embryos compete for the same resources in the brood pouch, and the winners are the ones with a better competitive edge, such as bigger

size (Ahnesjö, 1996; Partridge et al., 2009). However, this outcome may also be the result of cryptic male choice.

Cryptic male choice may work like it does in some other paternally caring species, where the male favours one female's eggs or offspring over others'. (Haig, 1990; Lessells, 2002). In some sense, this is also similar to differential allocation, which can generate sexual selection among favoured vs. disfavoured mates (Sheldon, 2000). In general, males may copulate longer with attractive females than unattractive females (Wang and Millar, 1997), or they may reduce sperm, nuptial gift and (as mentioned) parental care expenditure on less attractive females (Engqvist and Sauer, 2001). When males do this, they are also saving resources for future copulations, possibly with higher-quality females. Cryptic male choice in the broad-nosed pipefish could occur if males have a mechanism for providing more care to some embryos over others in the brood pouch, for example by supplying more oxygen or nutrients to the embryos of preferred females, or by removing the embryos of non-preferred females, through selective filial cannibalism (Ahnesjö, 1996; Klug and Bonsall, 2007). This thesis has shown that both processes could occur as I showed that multiple maternity broods had increased survival compared to single maternity broods, all other factors being equal (**paper II**). This effect may be caused by either increased paternal provision or less sibling competition in the multiple maternity broods.

Although I did not find an effect of male size on brood reduction or embryo weight in **paper II, III and V**, it has earlier been found that large males invest more energy per embryo than small males do (Berglund et al., 1986a). This is intriguing, because *S. typhle* males would have been expected to reduce their broods in order to promote offspring birth weight, especially because larger offspring size results in higher juvenile survival (Ahnesjö 1992a).

Parental care allocations may be affected by offspring number, since it affects both benefits and costs of offspring care. The degree of paternal care should also correlate with various other factors affecting the expected fitness returns from the current vs future broods, for example offspring genetic quality, diversity, age, as well as parent condition and future reproductive value (Clutton-Brock, 1991; Paczolt and Jones, 2010; Sheldon, 2000; Winkler and Wallin, 1987). Thus, males can invest more or less in the brood depending on the qualities in the brood, for instance, as found in **paper II** where I found less brood reduction when males brooded embryos of mixed maternity compared to when they brooded embryos of single maternity.

### **The energy based hypothesis and other reasons to perform brood reduction**

Mock and Parker (1997) postulated that the occurrence of brood reduction gives the parent a choice to adjust the size of the initial brood to current condition (resource tracking), and when performing brood reduction should keep the qualitatively best progeny (progeny choice hypothesis). I found both these mechanisms to fit with the broad-nosed pipefish system as male condition (**paper III, IV and V**) and egg size (**paper III and IV**) (Ahnesjö, 1996) affect brood reduction. I also found that numbers of eggs and density dependent egg

survival might affect brood reduction (**paper II and IV**) and that the energy based hypothesis probably plays a role of brood reduction in this species.

## **Future perspectives**

Parental conflicts and parent-offspring conflicts should be further investigated in the broad-nosed pipefish, since it provides an opportunity to separate processes acting through the egg and the brood pouch. I used this possibility to investigate questions concerning the benefits of multiple mating, brood size and male cost and benefits of paternal care.

### **Parental conflicts**

When only one parent invests in care and cares for more than one brood during its lifetime, conflict levels between parents are expected to rise since the parents may not have the same preference level of care performed (Parker, 1985). Parents may then try to manipulate the amount of care given through expression of genes in the offspring, hormones and behaviour. This parental conflict may then be expressed also as a parent-offspring conflict as both the non-caring parent and the offspring would prefer the caring parent to invest more into care. For instance, in offspring, genes are selected to promote the individual fitness. However, genes can have different fitness benefits or fitness costs depending on if the genes comes from the father or the mother (Haig, 1997).

In viviparous species with uni-maternal care and conventional sex roles, embryos that express the paternal genome can be selected to utilize more resources from maternal tissues, compared to the imprinting from the offspring's maternal genome (Haig, 1993). Placental hormones are also predicted to manipulate maternal (or paternal) physiology for foetal benefits. Genomic imprinting in mice has been shown to cause insulin-like growth factor II (IGF II) genes to be expressed in the embryo when coming from the father, but will not be expressed when donated from the mother, which is a sexually antagonistic trait. This may also cause conflicts between half-sibs during gestation (Haig, 1997). Major effects of imprinting on embryonic development may be largely restricted to viviparous species because actions that take place in an egg before hatching will usually have little direct effect on the parents or siblings in oviparous species. In eutherian mammals placental hormones are typically produced in much larger quantities than equivalent hormones in the non-pregnant state. This may be due to a conflict between foetal genes and maternal genes, where hormones are produced by the foetus to manipulate the nutrition provided by the mother (Haig, 1996). The possible differences in interest between males and females may result in a conflict over offspring development, growth and survival in the male brood pouch (Arnqvist and Rowe, 2005; Gil, 2003; Groothuis et al., 2005; Haig, 1990, 1993; Haig, 1996). Similar processes may take place in the broad-nosed pipefish. Here, the genetic conflict would be expressed as a female should try to manipulate the caring father into caring more for her offspring through maternally triggered genes and hormones deposited in the eggs. However, the caring male may avoid being manipulated by the hormones excreted by the

embryos by up- or down-regulating receptor density, to metabolize the “manipulating” hormone or to increase or decrease the parent’s own endogenous hormone production (Haig, 1993; Haig, 1996).

In this thesis I explore some adaptive ultimate causes for brood reduction in a pipefish. Still, the proximate mechanisms of brood reduction are not identified. For instance, I expect a genetic conflict to be present between the parents, as outlined above. This conflict should differ between species and populations depending on the level of brood reduction and multiple mating. However, in this thesis I have found several factors that influence the process of brood reduction. Brood reduction occurred through paternal absorption of nutrients emanating from embryos resembling filial cannibalism. Factors influencing the level of brood reduction were the number of female mating partners, brood size, egg size and male condition.

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