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Testes investment and spawning mode in pipefishes and seahorses (Syngnathidae)

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Externally fertilizing fishes are predicted to invest heavily in testes, because large numbers of sperm should be favoured by the high risk of sperm competition from sneaker males, and/or the dilution of ejaculates when shed into open water. Using museum specimens, we measured testes mass and body mass of 83 mature males, belonging to 21 genera of the family Syngnathidae (pipefishes and seahorses). In this family all species show paternal care, ranging in degree from eggs being attached to the skin of the male, to eggs being completely enclosed and nurtured within a brood pouch. The former, 'unprotected' group, is thought to have external fertilization, whereas in the latter, 'protected' group, males fertilize the eggs internally in their brood pouch. Smaller relative testes investment was thus predicted for genera that have protected compared with unprotected brood care. However, we found this not to be the case. Instead, all genera showed the same relationship between testes and body mass, regardless of brooding type. The possible implications of this surprising result are discussed, including the possibility that the mode of fertilization might have been misjudged for the pouchless syngnathids. *Biological Journal of the Linnean Society*, 2004, **83**, 369–376.

ADDITIONAL KEYWORDS: external fertilization – fish – gonadosomatic index – gonosomatic index – GSI – internal fertilization – Pisces – relative testes size.

INTRODUCTION

In many animal taxa, including fish, a positive correlation is found across species between the risk and/or intensity of sperm competition and investment into gonadal tissue; species with high risk and/or intensity of sperm competition generally have large testes relative to their body size (Stockley *et al*., 1997; Birkhead & Møller, 1998; Petersen & Warner, 1998; Byrne, Roberts & Simmons, 2002; but see Pyron, 2000). This pattern can be explained by an increased need for gonadal tissue required to produce the greater numbers of sperm that are favoured under sperm competition (Marconato & Shapiro, 1996; Parker, 1998).

By virtue of the fact that male parental care is relatively common among fish with external fertilization, it has been argued that external fertilization must be associated with low levels of sperm competition (or high certainty of paternity) (Trivers, 1972; Blumer, 1979; Perrone & Zaret, 1979). This is highly unlikely, however, because both group spawning and sneaking are common among externally fertilizing fish (e.g. Keenleyside, 1981; Taborsky, 1994; Petersen & Warner, 1998; DeWoody & Avise, 2001). Rather, external fertilization is likely to expose males to higher levels of sperm competition than internal fertilization (Petersen & Warner, 1998). For example Stockley *et al*. (1997) argued that the risk of sperm competition in internally fertilizing fish is likely to be reduced, compared with externally fertilizing species with the same level of polygamy or communal spawning, because of mortality of sperm between copulations or displacement of previous males' sperm during copulation. Thus, although sperm competition also occurs in internal fertilizers, as clearly demonstrated for example in guppies (Constantz, 1984; Evans & Magurran, 2001), the general pattern across species of fish is probably a

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lower level of sperm competition among internal fertilizers than external fertilizers.

There is a second reason why external fertilizers would be expected to have a greater investment in gonadal tissue than internal fertilizers. Among external fertilizers, sperm numbers can be severely limiting, and indeed are often insufficient to fertilize all of the eggs released during spawning, because sperm are rapidly diluted when shed into water (Petersen, 1991; Levitan & Petersen, 1995; Levitan, 1998). Byrne *et al*. (2002) found that, across species of externally fertilizing frogs, after controlling for sperm competition risk, species that shed sperm directly into the environment tended to have larger testes relative to their body size than did species spawning into foam nests. This pattern indicates that spawning environment can influence male investment in gonadal tissue. Fishes that spawn in open water often release large numbers of sperm, and consequently have large testes relative to their body size (Billard & Cosson, 1990). Thus, in general, external fertilizers are predicted to have larger testes than internal fertilizers. This is true even though fertilization rates lower than 100% can be adaptive for males (Warner *et al*., 1995; Ball & Parker, 1996; Shapiro & Giraldeau, 1996).

In the family of pipefishes and seahorses, the Syngnathidae, males always provide all post-zygotic parental care by carrying eggs (Breder & Rosen, 1966; Dawson, 1985). Despite the commonality of male pregnancy in the family, the methods of paternal brooding vary greatly between genera. At one extreme, eggs are simply attached to the skin of the caring male, as in the pipefish genera *Entelurus* and *Nerophis*, or they are attached to shallow egg compartments, as in the seadragons (*Phyllopteryx*) (Dawson, 1985). At the other extreme, eggs are not only protected, but also provided with oxygen and osmoregulation (Linton & Soloff, 1964; Dawson, 1985) and possibly also nutrients (Haresign & Shumway, 1981) in a highly specialized brood pouch, as is the case in pipefishes of the genus *Syngnathus* and among all seahorses (*Hippocampus*) (Dawson, 1985).

The particular way in which a male broods the young is also known to influence how eggs are fertilized. In genera with brood pouches, the female transfers the eggs at mating by inserting her ovipositor into the male's brood pouch. The male then fertilizes the eggs inside his own pouch (Fiedler, 1954; Boisseau, 1967, as cited in Lourie, Vincent & Hall, 1999). Because internal fertilization occurs within the male, the general theory regarding the relative expectations for sperm competition between internal and external fertilizing fishes outlined above does not strictly apply to seahorses and pipefishes. Rather, internal fertilization within the male makes these syngnathids free of the risks of sperm competition. Microsatellite DNA analyses of parentage in three species of pipefish with brood pouches, the dusky pipefish *Syngnathus floridae* (Jones, 1997), the Gulf pipefish *S. scovelli* (Jones & Avise, 1997) and the broad-nosed pipefish *S. typhle* (Jones *et al*., 1999), have revealed complete paternity within male broods. The same is true in the Western Australian seahorse, *Hippocampus subelongatus* (formerly *H. angustus*) (Jones *et al*., 1998; Kvarnemo *et al*., 2000). Thus, these syngnathids do indeed seem to be virtually free of sperm competition. Furthermore, the mode of fertilization inside the brood pouch is likely to have greater fertilization efficiency compared with external fertilization.

In contrast, for at least one species (*N. ophidion*) that lacks a brood pouch, it has been claimed that eggs are fertilized externally (Fiedler, 1954; Rosenqvist, 1993; McCoy, Jones & Avise, 2001). Although complete paternity has been shown among the broods of a relatively small sample of *N. ophidion* males (McCoy *et al*., 2001), some degree of sperm competition is theoretically possible where sperm are dispersed in water. Moreover, considerably more sperm would be needed to fertilize eggs in the external water column than within a brood pouch, because of their rapid dissipation (Levitan & Petersen, 1995). Hence, males of species that lack a brood pouch are expected to have relatively larger testes than males of species having a brood pouch.

The aim of our study was to examine male investment in gonadal tissue across genera of sygnathids. Our prediction was that syngnathids possessing a brood pouch should have smaller testes in relation to their body size than syngnathids that lack a brood pouch. Our prediction is based on the argument that greater numbers of sperm are required when fertilizing eggs externally, whether through increased risk of sperm competition (Parker, 1998) or because of sperm limitation in water (Levitan, 1998).

MATERIAL AND METHODS

We dissected 83 sexually mature males of 38 species of pipefishes and seahorses, belonging to 21 different genera within the family Syngnathidae. All animals had been preserved in alcohol. The majority of our data came from specimens in the collections of the Western Australian Museum, Perth, Australia. In addition, material was included that originated from five other collections (Florida Department of Environmental Protection/Florida Marine Research Institute, St Petersburg, Florida, USA; Museo di Storia Naturale e del Territorio, University of Pisa, Italy; the Royal Ontario Museum, Toronto, Canada; Biology Department, University of Papua New Guinea, Papua New Guinea) as well as a few specimens collected by Ingrid Ahnesjö and Charlotta Kvarnemo, Sweden. We

dissected all specimens under standard light microscopy using 60–120× magnification. After removal of the testes, body weights were taken to the nearest 0.001 g, using a digital balance. All weights were taken as wet weights, after carefully removing excess moisture. Testes were weighed to the nearest 0.001 mg on a Cahn Micro Balance. Only males that were clearly reproductively mature were included in the study. We determined this from the presence of eggs or, if no eggs were present, from marks of recent eggs or the developmental state of the brood pouch or brooding area which are fully developed only during the breeding season (Dawson, 1985). When eggs were present, their extra weight was removed from the body weight by weighing one egg and multiplying its weight by the number of eggs. Specimens for which we could not reliably count the number of eggs were not included. In addition, for *N. ophidion*, a pouchless species for which maturity is difficult to determine in the absence of eggs, four unmated live males, which were seen actively courting prior to capture, were killed. These fish were preserved in 70% alcohol for 10 months, before being dissected.

Alcohol preservation may cause shrinkage of tissues so that our measures of body and testes weights may represent underestimates of the true absolute species values. Nevertheless, as preservation methods were similar for all species examined, our measures should serve as adequate relative estimates of body and testes weights across the species examined. Byrne *et al*. (2002) likewise used alcohol-preserved material in their comparative analysis of testes investment across species of frogs. Their preliminary comparisons of fresh and alcohol-preserved material suggests that alcohol preservation does not significantly bias weight measurements (Byrne *et al*., 2002).

Following Herald (1959), Dawson (1985) and Wilson *et al*. (2001, 2003), we categorized the brood types found among the species of Syngnathidae according to position (A = tail or B = trunk) and morphology (1–5 with increasing complexity), resulting in eight existing combinations (A1 and B5 are non-existent). We divided these eight brooding types into two main categories: males having an unprotected or a protected brood pouch at mating. The unprotected group consisted of genera in which eggs are attached to the skin of the trunk of the male (B1), or into membranous egg compartments on the skin (A2 or B2). The protected group consisted of genera that protect the eggs by pouch plates (A3 or B3), pouch folds (A4 or B4) or in a sac (A5). However, we made one exception. We categorized the genus *Corythoichthys* as unprotected, despite it having a brood pouch (A4), as the pouch is formed from thin folds that are not raised until after mating (Kuiter, 2000). Furthermore, in the genus *Halicampus*, representatives of more than one brood type were found (Table 1). In our analysis, we used these as independent data points, i.e. we entered the genus twice, once for each brood pouch type. We based this decision on the fact that, according to Dawson (1985: 1 and 77), the genus *Halicampus* is likely to be polyphyletic and *H. macrorhynchus* probably belongs to a separate genus. The unprotected brood pouch that we noted in our single specimen of *H. macrorhynchus* can be confirmed from a photograph in Kuiter (2000: 163). However, excluding the data point of *H. macrorhynchus* does not change any of our conclusions.

Despite recent phylogenetic studies of Syngnathidae (Wilson *et al*., 2001, 2003), we do not have a phylogeny that adequately covers the species of syngnathids for which we have testes data. Therefore, in addition to reporting the result using species means, we attempt at least partially to control for common ancestry in our analysis by using generic means (Crook, 1965; Harvey & Pagel, 1991). The generic means were calculated from species mean values. Including the two estimates of *Halicampus*, our sample size is 22, nine of which we have categorized as unprotected and 13 as protected. We log-transformed all our data to control for allometric scaling and to achieve normality. We then analysed testes weight using a one-factor analysis of covariance, with body weight as the covariate and level of protection as a factor (unprotected or protected). For comparison, we also report the result using level of protection 1–5 as a factor (cf. above). However, owing to few genera having brood care of levels 1 or 5, this analysis could not be done on genus mean values. Analysing relative testes weight using analysis of covariance is preferred to the more commonly used gonosomatic index (GSI = 100 × testes weight/body weight) (Tomkins & Simmons, 2002). However, again for comparison, we also report the result using GSI.

RESULTS

The mean values of testes weight and body weight were calculated for each species (Appendix) and genus (Table 1). Across genera testes mass was highly dependent on body mass, but not on the level of brood pouch protection (one-factor ANCOVA; covariate (body weight): $F_{1,19} = 145$, $P < 0.001$; factor (protected or unprotected): $F_{1,19} = 0.014$, $P = 0.91$) (Fig. 1). The interaction term between the covariate and the factor was non-significant ($F_{1,18} = 0.10$, $P = 0.75$) and therefore deleted from the analysis. Essentially the same result was found when species mean values where used (one-factor ANCOVA; covariate (body weight): $F_{1,36} = 181$, $P < 0.001$; factor (protected or unprotected): $F_{1,36} = 0.085$, $P = 0.77$; interaction: $F_{1,35} = 0.026$, $P = 0.87$) and when all five levels of brood

Table 1. Mean values of testes wet weight and body wet weight for 21 genera of the family Syngnathidae, based on the species means. Brood type refers to the position (A = tail or B = trunk) and the morphology (1–5 with increasing complexity) of the brooding area; category refers to our division into unprotected or protected brood care based on the absence or presence of a brood pouch at mating; Twt, testes wet weight (mg); Bwt, body wet weight without testes (g); *N*, number of specimens; the species names are those species that are represented in our samples (superscript numbers indicate the origins and identification codes given in the footnote)

Genus	Brood type	Category	Twt	Bwt	*N*	Species names
Choeroichthys	B4	protected	0.84	0.17	3	*brachysoma*[1]
Corythoichtys	A4	unprotected	2.31	1.41	9	*flavofasciatus*[2], *haematopterus*[3], *intestinalis*[4], *sp.*[5]
Cosmocampus	A4	protected	11.70	8.07	1	*albirostris*[6]
Doryrhamphus	B2	unprotected	0.08	0.06	3	*negrosensis*[7]
Dunckerocampus	B2	unprotected	2.51	1.70	7	*dactyliphorus*[8]
Entelurus	B1	unprotected	1.23	0.44	1	*aquareus*[9]
Festucalex	A4	protected	3.08	2.50	5	*scalaris*[10]
Filicampus	A4	protected	9.72	5.87	4	*tigris*[11]
Halicampus	A4	protected	2.65	0.82	6	*brocki*[12], *grayi*[13], *nitidus*[14]
Halicampus	A2	unprotected	6.42	2.60	1	*macrorhynchus*[15]
Haliichtys	A4	protected	34.71	10.28	1	*taeniophora*[16]
Heraldia	B2	unprotected	5.09	0.52	1	*nocturna*[17]
Hippichtys	A4	protected	0.94	0.52	5	*heptagonus*[18]
Hippocampus	A5	protected	13.12	11.52	7	*angustus*[19], *erectus*[10]
Microphis	B3	protected	2.17	1.04	3	*brachyurus*[21], *mento*[22], *retzi*[23]
Nannocampus	A4	protected	1.12	0.39	1	*subosseus*[24]
Nerophis	B1	unprotected	1.42	0.36	6	*ophidion*[25]
Phyllopteryx	A2	unprotected	31.57	14.42	2	*taeniolatus*[26]
Solegnathus	A2	unprotected	35.70	43.48	2	*lettiensis*[27], *spinosissimus*[28]
Stigmatophora	A4	protected	1.85	0.94	3	*argus*[29], *nigra*[30]
Syngnathus	A4	protected	10.70	5.24	10	*abaster*[31], *acus*[32], *floridae*[33], *fuscus*[34], *rostellatus*[35], *scovelli*[36], *springeri*[37], *typhle*[38]
Vanacampus	A4	protected	4.93	3.29	2	*poecilolaemus*[39]

[1]WAM 31305-22, 31305-51; [2]ROM 35966, 36969, 38905; 46 : 1G; [3]WAM 30405-12; [4]WAM 30118-1, UPNG 262, 509; [5]PISA 2389/2; [6]FL 01977; [7]WAM 30412-3, 27828-5; [8]ROM 43994, 43983, 44006, WAM 30618-10, 28174-29, 31140-13; [9]PISA 510; [10]WAM 26471-1, 30164-1, 30166-2, 30168-1, 30681-3; [11]WAM 26478-1/560, 26478-1/562, 26478-1/563, 26491-1; [12]WAM 27955-4, 27957-9; [13]WAM 26492, 28763-1; [14]WAM P28021-9, 30684-5; [15]WAM 28026-5; [16]WAM 85380; [17]WAM P25799-1; [18]WAM P26957-12, P27776-4; [19]WAM P6067, P14738, P25080-2, P26053-1, P27351-1; [20]FL 03781/15862; [21]WAM 31037-2; [22]WAM 29603-14; [23]WAM 28164-13; [24]WAM 27950-12; [25]CK 1, 2, 3, 4; PISA 2912/12, 2912/7; [26]WAM 2995-1, 29677-1; [27]WAM 29074-1; [28]WAM 29082-1; [29]WAM 26455-1; [30]WAM 25346-12, 29072-1; [31]PISA 1454/1; [32]IA 45:B; [33] FL 00905; [34] ROM 04372; [35]IA 45:B; [36]FL 00539/07823; [37]FL 10253/10954; [38]PISA 679/1; [39]WAM 5886–1, 28363–5. Museum collections: FL, Florida Department of Environmental Protection, Florida Marine Research Institute, St Petersburg, USA; PISA, Museo di Storia Naturale e del Territorio, University of Pisa, Italy; ROM, the Royal Ontario Museum, Toronto, Canada; UPNG, Biology Department, University of Papua New Guinea, Papua New Guinea; WAM, Western Australian Museum, Perth, Australia. In addition, CK = Charlotta Kvarnemo, Department of Zoology, Stockholm University, Sweden, and IA = Ingrid Ahnesjö, Department of Evolutionary Biology, Uppsala University, Sweden. These specimens were collected nearby the islands Skaftö and Gåsö, at the Swedish west coast, in May 2002 and 1994, respectively.

care were used as the factor, rather than just protected or unprotected (one-factor ANCOVA; covariate (body weight): $F_{1,33} = 150$, $P < 0.001$; factor (brood care 1–5): $F_{4,33} = 0.26$, $P = 0.90$; interaction: $F_{4,29} = 0.42$, $P = 0.79$). Similarly, the result did not change when the analysis was based on the genus mean GSI. Thus, genera that have a protected brood pouch did not differ from unprotected genera in their GSI (one-factor ANOVA; $F_{1,20} = 0.15$, $P = 0.71$). The mean GSI across all genera, based on the genus mean values of testes weight and body weight presented in Table 1, was 0.254 ± 0.192 (SD).

The use of generic means has its limitations because they can inflate the degrees of freedom and generate relationships that are due to taxonomic or phylogenetic affiliation at higher nodes in the phylogeny (Harvey & Pagel, 1991). However, from our data we are able to conclude that there is no covariation between

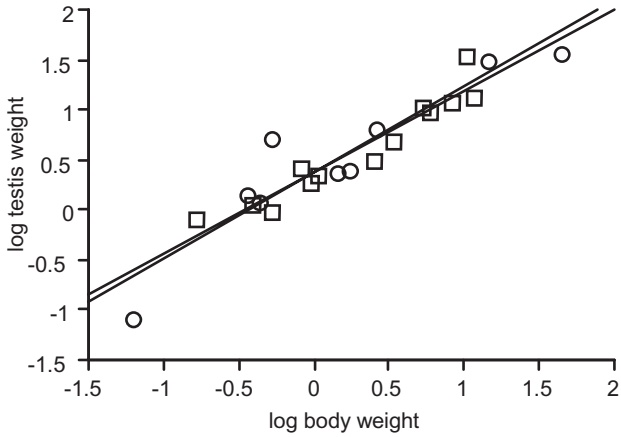

Figure 1. The relationship between log transformed genus mean values of body wet weight (g) and testes wet weight (mg) did not differ between genera with protected and unprotected brood care (i.e. brood pouch present or absent). □, protected; ○, unprotected.

the traits of interest because although common ancestry can potentially generate relationships between two variables, it cannot obscure such relationships (Harvey & Pagel, 1991).

DISCUSSION

In this paper we investigated whether the males of syngnathids that fertilize and brood their eggs protected within a brood pouch have smaller relative testes size than the males of species that fertilize and brood their eggs externally. To our surprise we did not find any difference in testes size between these groups. We based our prediction on the argument that males will need greater numbers of sperm, and therefore larger testes, when fertilizing eggs externally. This prediction should hold whether primarily due to an increased risk of sperm competition (Parker, 1998) or to a lower fertilization efficiency (Levitan & Petersen, 1995; Levitan, 1998) among externally fertilizing syngnathids. Hence, the lack of difference in testes investment between the genera that belong to the protected and the unprotected categories suggests that one or more assumptions must be reconsidered.

Previous comparative studies of fish have shown that external fertilization and sperm competition are associated with increased testes size (Stockley *et al*., 1997; Petersen & Warner, 1998). Although external fertilization need not always be associated with sperm competition from sneaking males (e.g. DeWoody *et al*., 2000), when it is, the means by which males increase their fertilization success in the face of sperm competition need not always be simply an increase in the number of sperm produced. Competitive fertilization success can also be increased by the swimming speed and/or longevity of sperm, which may in turn be a function of sperm length and/or energy reserves (Ball & Parker, 1996; Vladic′ & Järvi, 2001; Vladic′, Afzelius & Bronnikov, 2002). We would then predict longer sperm and/or increased motility in sygnathid species that fertilize their eggs externally, compared with those having a brood pouch. At present, there are not enough data avaliable to test this possibility. However, comparing the morphology and longevity of sperm of one representative species from each group, *Nerophis ophidion* (Œ. Ah-King, H. Elofsson, C. Kvarnemo, G. Rosenqvist & A. Berglund, unpubl. data) and *Syngnathus schlegeli* (Watanabe, Hara & Watanabe, 2000), suggests that sperm characteristics are remarkably similar.

Regardless of the importance of sperm competition for testes size variation across the Syngnathidae, there are solid grounds for expecting external fertilization to require a high expenditure on sperm production (Billard & Cosson, 1990; Levitan & Petersen, 1995). However, the GSI across a wide range of other externally fertilizing fish families ranges from 0.3 to 12 (see Stockley *et al*., 1997), most of which are orders of magnitude higher than the average GSI of 0.25 reported here for syngnathids. Similarly, limiting the comparison to the relatively closely related family Gasterosteidae, the GSI values for the peak of the breeding season are clearly higher than those of the syngnathids (e.g. *Eucalia inconstans* 1.0–1.1: Ruby & McMillan, 1970; *Gasterosteus aculeatus* 2.0–2.1: Chellappa *et al*., 1989; Huntingford, Chellappa & Taylor, 2001; *Spinachia spinachia* 0.50: M. Páll, unpubl. data). Thus, the extremely low testes weights found across the entire family of syngnathids seem to contradict the claim that some species exhibit external fertilization. Consequently, there might be reasons to question whether males that lack a brood pouch do in fact fertilize the eggs in open water.

In species that lack a brood pouch, detailed information on mode of fertilization is limited to just one genus, *Nerophis*. Fiedler (1954) described the mating in *N. ophidion* in great detail. In translation from the German original (p. 378), he wrote that ‘Apparently, while the male twists around the female he ejaculates the sperm and distributes it over the eggs. This was not visible to the naked eye’. This description has later been re-interpreted as if external fertilization is a documented fact in this species. For example, Rosenqvist (1993) and McCoy *et al*. (2001) both refer to Fiedler (1954) when stating that in *N. ophidion* the male fertilizes the eggs while sinking through a cloud of sperm that has been ejaculated into the water. However, to our knowledge there exists no firm evidence that *N. ophidion* actually fertilizes its eggs externally, in the sense of a sperm cloud being released into the open

water, and indeed the low GSI for this species suggests that this type of spawning is unlikely.

In their genetic study of *N. ophidion* broods, McCoy *et al*. (2001) found only one case that could possibly be interpreted as a male carrying an embryo it had not fathered, which suggests a very low level of sperm competition in this species. Based on the fact that *Nerophis* females attach the eggs to the male starting from the anal opening (Fiedler, 1954; Monteiro, da Natividade Vieira & Almada, 2002), alternative modes of fertilization have recently been investigated for *N. lumbriciformis* (Monteiro *et al*., 2002) and for *N. ophidion* (Œ. Ah-King *et al*., unpubl. data). The authors of the former study discuss the possibility that sperm might be pushed forward from the genital area of the male as the female attaches the eggs to the male body, whereas the result of the latter study suggests that the eggs might even be fertilized internally, as sperm were found within the genital tract of newly mated females. For example, it is possible that the female copulates briefly with the male the moment she touches the male's anal opening at the start of egg transfer, and that the eggs would be fertilized as they are laid. Such alternative models of fertilization would require very small amounts of sperm, are likely to provide protection against sperm competition from sneaker males, afford more efficient fertilization than the sperm cloud hypothesis and hence be more consistent with the low GSI observed in this species.

It is still unknown whether all the pouchless syngnathids fertilize their eggs externally. Yet, reconsidering the mode of fertilization appears to us to be the most parsimonious way to explain the widespread pattern of minimal testes investment among these fish. Otherwise, we need to invoke suites of explanations for why external fertilization should be associated with neither an increased risk of sperm competition nor lower fertilization efficiency. Regardless, further work will be needed to understand fully and explain the surprising results uncovered in this present study.

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APPENDIX

Basic statistics (count, mean, standard deviation and range) of testes wet weight (twt, measured in mg) and body wet weight (bwt, measured in g) for each of the species of syngnathids that were included in our study.

Species	*N*	Mean twt	SD	Min.	Max.	Mean bwt	SD	Min.	Max.
Choeroichthys brachysoma	3	0.84	0.39	0.57	1.29	0.17	0.02	0.15	0.19
Corythoichtys flavofasciatus	4	1.22	0.86	0.34	2.40	0.67	0.21	0.37	0.85
Corythoichtys haematopterus	1	2.32				1.66			
Corythoichtys intestinalis	3	2.04	1.72	0.09	3.35	1.32	0.70	0.53	1.87
Corythoichtys sp.	1	3.64				2.00			
Cosmocampus albirostris	1	11.7				8.07			
Doryrhamphus negrosensis	3	0.08	0.07	0.02	0.16	0.06	0.03	0.03	0.08
Dunckerocampus dactyliphorus	7	2.51	1.67	0.86	5.08	1.70	1.06	0.51	3.09
Enterlurus aquareus	1	1.23				0.44			
Festucalex scalaris	5	3.08	1.26	1.80	4.96	2.50	1.06	1.08	3.88
Filicampus tigris	4	9.72	4.05	4.26	13.77	5.87	1.15	4.78	7.47
Halicampus brocki	2	2.10	1.30	1.18	3.02	0.48	0.16	0.36	0.59
Halicampus grayi	2	5.61	0.04	5.58	5.63	1.76	0.75	1.23	2.29
Halicampus macrorhynchus	1	6.42				2.60			
Halicampus nitidus	2	0.23	0.27	0.04	0.42	0.23	0.13	0.14	0.32
Haliichtys taeniophora	1	34.7				10.3			
Heraldia nocturna	1	5.09				0.52			
Hippichtys heptagonus	5	0.94	0.44	0.55	1.68	0.52	0.11	0.40	0.64
Hippocampus angustus	5	18.3	9.10	6.93	31.0	9.65	2.13	7.92	13.0
Hippocampus erectus	2	7.90	4.38	4.80	11.0	13.4	3.03	11.2	15.5
Microphis brachyurus	1	2.24				1.65			
Microphis mento	1	3.55				1.08			
Microphis retzi	1	0.73				0.41			
Nannocampus subosseus	1	1.12				0.39			
Nerophis ophidion	6	1.42	0.50	1.06	2.34	0.36	0.06	0.31	0.45
Phyllopteryx taeniolatus	2	31.6	12.9	22.5	40.7	14.4	5.38	10.6	18.2
Solegnathus lettiensis	1	15.5				27.0			
Solegnathus spinosissimus	1	55.9				60.0			
Stigmatophora argus	1	3.36				1.76			
Stigmatophora nigra	2	0.34	0.34	0.10	0.58	0.13	0.01	0.12	0.13
Syngnathus abaster	1	1.17				0.46			
Syngnathus acus	1	52.4				21.5			
Syngnathus floridae	2	1.02	1.10	0.25	1.80	0.71	0.46	0.39	1.04
Syngnathus fuscus	2	4.67	3.34	2.30	7.03	1.57	1.03	0.84	2.30
Syngnathus rostellatus	1	7.29				0.47			
Syngnathus scovelli	1	0.76				0.61			
Syngnathus springeri	1	15.0				13.9			
Syngnathus typhle	1	3.34				2.68			
Vanacampus poecilolaemus	2	4.93	1.87	3.61	6.25	3.29	1.63	2.14	4.45
Total	83	6.44	10.8	0.02	55.9	4.07	8.08	0.03	60.0