What Determines the Minimum Body Size for Vertebrates?

Klaus M. Stiefel[1,2] & Abner A. Bucol[3]

1. Neurolinx Research Institute, La Jolla, CA, USA

2. Silliman University Institute of Environmental & Marine Sciences, Dumaguete City, Philippines

3. Silliman University Angelo King Center for Research & Environmental Management, Dumaguete City, Philippines

Abstract

The minimum body size of vertebrate species lies just above 6 millimeters, in stark contrast to the minimum sizes attained by species of other major taxonomic groups. This paper presents two connected hypotheses explaining this minimum size obtainable with a vertebrate Bauplan. Firstly, the complex bodies of vertebrates might not be amendable to reduction below a certain level of complexity. We hypothesize that this lower limit for organ complexity hold especially true for the vertebrate body's most complex organ, the brain. This is at least partially due to poor scaling of the brain to small sizes, and a coding strategy known as population coding.

In the context of poor scaling of a complex Bauplan, we discuss the relative sparsity of paedomorphism and parasitism in vertebrates.

Second, these constrains will disproportionately affect the smaller bodies and brains of juvenile or larval animals. This, in turn requires a certain minimum egg size to for a juvenile or larva to reach a size where it can independently function. Due to that minimum egg size, the number of eggs per female will decrease with decreasing female body size, indirectly limiting adult size. That problem is likely aggravated since extremely small animals are likely to be low in the food web and in need of high reproductive rates to offset high mortalities caused by predation. Hence, below a certain body size a female will not be able to produce enough eggs to reach the population replacement value. We demonstrate the scaling relationships relevant for this argument with data from gobiid fishes.

The first argument is about animal body complexity; the second argument stems from ecology.

Introduction

Animal body size varies over many orders of magnitude, in vertebrates ranging from the blue whale (*Balaenoptera musculus*) at up to 30 meters and 170 tons down to several species less than a centimeter in length. Minute species are found among several different vertebrate lineages (Fig. 1, Tab. 1, and references in Tab. 1; Fig. 2). Teleost (bony) fishes gave rise to several very small species, including the Philippine freshwater dwarf goby *Pandaka pygmaea* at 7 mm, the highly paedomorphic marine genus *Schindleria* at down to 6.5 mm, *Trimmatom nanus* at 10 mm and the Ind-Pacific marine genus *Eviota* at 8 mm. These dwarf gobies of the genus *Eviota* are also the vertebrates with the briefest known life-spans, at less than 100 days (Depczynski & Bellwood, 2006). The sexually parasitic males of the deep-sea anglerfish *Leptophilypnion pusillus* reach only 6.5 mm, and the cyprinid *Paedocypris progenetica* only 7.9 mm. The Papua New Guinean leaf-litter frog *Paedophryne amauensis* grows to an average adult size of 7.7 mm (Rittmeyer et al., 2012). Several evolutionarily distinct lineages of miniaturized frogs from South America (Taucce et al., 2020), India (Biju et al., 2007), and the Seychelles are known, only slightly larger than *P. amauensis*. Smallest among the amniotes, the miniaturized gekkonid lizard *Sphaerodactylus ariasae* reaches an average size of only 16 mm (Hedges & Thomas 2001).

This aforementioned species are examples of miniaturized species and the listing is not intended to be complete. Additionally, due to their small size, often cryptic habitats and occurrence in hard-to-reach parts of the world like tropical rain forests, new miniaturized vertebrate species are still regularly being discovered (Taucce et al., 2020). Interestingly, none of these miniaturized vertebrate species are smaller than 6 mm.

This is in stark contrast to the smallest members of many other phyla (Fig. 2). For instance, the smallest insects are parasitic wasps of the genus *Dicopomorpha*, at 0.139 mm adult size (Mockford 1997); the smallest crustacean is *Stygotantulus stocki* at 0.094 mm (Martin & Davis 2001), and the smallest known gastropod is *Ammonicera minortalis* at 0.32 mm (Bieler & Mikkelsen, 1998).

These above species are examples from phyla with complex Baupläne (organismal organization). Comparably simpler animals can reach even smaller sizes, the highly reduced parasitic cnidarian *Myxobolus shekel* reaches an adult size of only 89 μm and was initially even mistaken for a protist (Kaur et al., 2016; Fig. 2).

The ratio in length between a blue whale and the smallest known fish (~3900:1) is only about four times larger than the ratio between the smallest known fish and the smallest known metazoan, the aforementioned cnidarian *Myxobolus shekel* (~900:1, Fig. 2). The aim of this paper is to explain the stark difference in the lower size limit between vertebrates and other lineages of animals by using theoretical arguments about the organismal organization of animals, from neurobiological and ecological perspectives.

Species	**Taxonomic group**	**Adult size (mm)**	**Comment**	**Reference**
Sphaerodactylus ariasae	Chordata/Sphaerodactylidae	16		Hedges & Thomas, 2001
Trimmatom nanus	Chordata/Gobiidae	10		Winterbottom, 1990
	Chordata/Elotrelidae	8.4	parasitic	Roberts, 2013
Pandaka pygmaea	Chordata/Gobiidae	9		Herre, 1929
Eviota queenslandica	Chordata/Gobiidae	8	life span < 100 days	Depczynski & Bellwood, 2006
Paedocypris progenetica	Chordata/Cyprinidae	7.9	pedomorphism	Kottelat et al., 2006
Paedophryne amanuensis	Chordata/Anura/ Microhylidae	7.7	pedomorphism	Thompson et al., 2012
Schindleria brevipinguis	Chordata/Gobiidae	6.5	pedomorphism	Watson & Walker, 2004
Idiosepius notoides	Molusca/Cephalopoda	6		Tracey et al., 2003
Ammonicera minortalis	Molusca/Gastropoda	0.32		Bieler & Mikkelsen, 1998
Dicopomorpha echmepterygis	Arthropoda/Insecta	0.139	parasitic	Mockford, 1997
Stygotantulus stocki	Arthropoda/ICrustacea	0.094		Martin & Davis, 2001
Myxobolus shekel	Cnidaria	0.0085	parasitic	Kaur et al., 2016

Table 1: Exemplary miniaturized vertebrate (black) and invertebrate (red) species and their lengths.

Methods

The conclusions of this paper are derived from theoretical arguments presented below.

Several measures of animal size exist, however especially in diminutive species weight is difficult to determine and is often not available. In fishes (without significant weight in their limbs), weight systematically relates to length as $w = a\, l^b$, with a and b depending on the fish body type (Froese et al., 2014). For the sake of simplicity, we use length as the measure of animal size in this paper.

Results

We present a hypothesis to explain why no vertebrate species attain minimum adult sizes below about 6 millimeters. The hypothesis has two parts, the first one arguing that the complex bodies of vertebrates impose a lower size limit. This limit exists especially due to the complexity of vertebrate brains, the most complex organs of vertebrate animals. Furthermore, brain size is at least partially limited by its over-proportional scaling at small sizes, it's use of a neural coding strategy called population coding, and its over-proportional energy use.

The second part of the hypothesis argues that these factors primarily limit sizes of the smaller juveniles or larvae of diminutive species. In order to produce an independently functioning juvenile or larva of a minimum size, a minimum egg size will be necessary. This minimum egg size together with the minimum number of eggs necessary for population replacement puts a lower limit on the size of females.

For a schematic overview over our arguments, see Fig. 4.

Limit by Bauplan

A Bauplan is a suite of characters shared by a group of phylogenetically related animals at some point during their development. The concept was as first introduced by Joseph Henry Woodger in 1945 (Willmore, 2012).

Animal Baupläne (German plural of Bauplan) vastly differ in their complexity, from sponges with only a small number of cell types, and a limited set of patterns and rules governing the arrangements and interactions, to highly evolved lineages like vertebrates, mollusks and arthropods with multiple finely structured and precisely coordinated tissues and organs. While there is clearly a gradient in complexity from a sponge to a human, it's no trivial matter to measure organismal complexity. The complexity of interactions and organizing principles is difficult to quantify (McShea 1992; Tenaillon et al., 2007).

The number of different cell types is a useful measure of the complexity of an animal. This, again, is no trivial measure, since cell types are not clearly and obviously delineated. Cells might be classified according to morphology, physiology or gene expression patterns, and these classifications might or might not overlap. Nevertheless, an estimate of cell types on a coarse scale is feasible.

Valentine et al. (1994) estimate that cnidarians contain 11 types of cells, *Drosophila* contains ~50 types, and zebrafish (*Danio rerio*) and humans contain ~150 types of cells. In the body of an animal every type of cell will be present in multiple instances, and we can not simply interpolate that an animal with three times the number of cell types will be at least three times larger. Rather, the number of cell types serves as a proxy for complexity, and an approximately three-fold increase in cell type number corresponds to a significant increase in organismic complexity. Based on these differences in cell type numbers we propose that the high level of organismic complexity in the vertebrate Bauplan is the *primary* reason preventing vertebrates from attaining body sizes of less than 6 millimeters.

However, a more complex system (animal body) with more different components (cell types) will inevitably be larger. Each cell type will be represented in the body of an animal not once, but multiple times, forming tissues and organs. A larger number of individual components alone will take up more space; An increased number of parts will also lead to an increasing number of combinations of these parts, and more complex tissues and organs, in turn taking up more space again. For each tissue there will be a lower size limit for proper function; more cell types, and hence more tissues and organs will increase that lower limit. Figure 2 shows that the numbers of cell types estimated by Valentine et al. (1994) at least coarsely correlate with the minimum body sizes of various phylogenetic groups.

Limit by Bauplan – Brain Complexity

The argument presented above that the vertebrate Bauplan is more complex than that of other lineages and hence sets a lower size limit for vertebrate body size is most pressing when applied to the most complex organ of the vertebrate body, the brain. We propose that specifically the complexity of the vertebrate brain is what limits the smallest sizes vertebrates can attain. This argument is supported by several lines of inquiry, specifically into the number of neuronal cell types, the scaling of the brain, energy use by the brain, and the nature of neural coding in vertebrates.

As with the number of primary cell types in bodies, the number of sub-types of neurons can serve as a reasonable proxy for the complexity of the organ. Again, this is no trivial measure, and morphological, physiological and gene-expression classifications of neurons might not overlap. Recent decades have seen a wealth of studies of the neural types in vertebrate brains, especially in the mammalian cortex, where especially the class of the inhibitory interneurons is highly diverse in form and function (Buzsáki et al., 2004; DeFelipe et al., 2013). Expert opinion is still split over the exact number of interneurons in the brain of the rat (a popular model organism in neuroscience) and about the preferred classification scheme (based on a combination of physiological, anatomical, and gene-expression data), but at least 20 sub-types are thought to exist.

Even the principal (pyramidal) neurons, long thought to be a rather monolithic class, have recently been shown to consist of 19 sub-classes in the rat somatosensory cortex alone (Kanari et al., 2019). This are but only two classes of neurons, in only one brain structure of a rodent. No comprehensive listing of all known neuron sub-types in a vertebrate is available to our knowledge, but we can safely assume a number of more than 100.

There are no comparably extensive studies of neuronal cell types in insects, however some work has been done in *Drosophila*, where gene expression studies identified just below 30 cell types for the whole brain (Crosset et al., 2017). The *Drosophila* brain, with $\sim 10^5$ neurons and multiple distinct regions, is undoubtedly a complex information processing organ capable of coordinating precise flight and complex courtship rituals but is clearly below mammalian brains in terms of neuron types. Still, its complexity is lower when compared to a rat brain with $\sim 2\ 10^8$ neurons (Herculano-Houzel & Lent, 2005) and several times the number of neuron types. The arguments raised above, that a more complex system has a larger minimum size to which it can be downscaled to holds especially for vertebrate brains.

A second point also heightens the importance of the brain for determining the larger minimum size of vertebrates. Nervous systems scale with different allometric coefficients than the rest of the body, with brains and sensory organs being relatively enlarged to the rest of the body in miniaturized species (Striedter, 2005). The smaller an animal gets, the higher it’s brain/body-weight ratio gets. Since these allometric scaling curves have a higher offset for vertebrates (which have larger brains per body weight) they intersect the y-axis earlier; and the point where the large relative brain size is so costly that it’s no longer feasible is reached at larger body sizes.

A third point, which is related to the previous point is that energy use scales inversely with body size (Speakman, 2005). This is not only due to energy use by the brain, however the brain uses disproportionally much energy. The “expensive tissue hypothesis” suggests that the heavy metabolic cost of a large brain has to be offset by reducing other organ systems (Kotrschal et al., 2013; Liao et al., 2016), and this offset will only be feasible to a certain extent, which will also delimit vertebrate minimum body size.

Finally, a fourth point relates to the type of neural coding which is widely used in vertebrate brains, and might not perform well at very small brain sizes. Population coding is the principle of encoding information via the activities of large numbers of neurons, where each perceived sensory stimulus or planned motor program is encoded via a distribution of neural activities in a population of neurons. Population coding does exist in invertebrates, at least in insects, where it is well established in the insect olfactory bulb (Stopfer et al., 2003). In contrast, the ganglia of simpler invertebrate nervous systems such as the well-researched examples in the leech, lobster, or sea slug (*Aplysia*), rely on coding by individual neurons or small groups of neurons and do not employ population coding.

Nevertheless, population coding is almost certainly more prominent in vertebrates, where large neuron populations in orderly (cortical) neural structures are ideally suited for such a coding strategy. Theoretical arguments suggest population coding scales poorly to small numbers of neurons. In simulations, the information content of a network using population coding rapidly dropped to a fraction of its maximum when the total neuron number dropped below ~100 (Sompolinsky et al., 2001). The absolute number of neurons necessary for functioning population coding undoubtedly depends on a variety of factors, such as the intrinsic noise of the nervous system (which also increases with small sizes), but the conclusion that large numbers of neurons are necessary for this type of neural coding is solid. This necessity to maintain a larger minimum number of neurons to allow for this coding style will also limit the minimum possible brain size.

Interestingly, the behavioral repertoire of minute spiders does not seem to be reduced compared to larger related species (Eberhard, 2007). We are not aware of comparable studies for miniaturized and related larger vertebrates (such as cyprinid fishes). A prediction from the arguments presented here is that for vertebrates (using population coding more prominently, see above), performance *would* decrease at minute body sizes.

Parasitism and Paedomorphism in anatomically reduced Miniature Animals

In the context of the reduction of bodily complexity it is noteworthy that only a few anatomically reduced vertebrate parasites exist, and that paedomorphism is typically limited in vertebrates.

Parasitism often goes hand in hand with a reduction of bodily complexity of all organ systems but for the reproductive apparatus. This is very rarely seen in vertebrates. Rather, the few known vertebrate parasites have typically adapted their behavior and not their anatomy to parasitism. One example is the cookie-cutter shark, an ectoparasite of cetaceans which remains a free-swimming fish (Dwyer & Visser, 2011). The cuckoos among the birds and the catfish *Synodontis multipunctatus* (Sato, 1986) are brood parasites, which trick other birds (in the case of the cuckoos) and cichlid fishes (in the case of the catfish) into protecting (and feeding in the case of the cuckoo) their offspring. The remoras are hydrodynamic parasites which temporarily attach to larger fishes and use their locomotory effort for movement and for the transport of oxygenated water over their gills. Most of these vertebrate parasites parasitize services instead of nutrients, and none of them have significant reductions of the complexity of their bodies as a consequence of their parasitic lifestyles.

An exception in terms of reduction of bodily complexity are the males of certain deep-sea anglerfish species, which upon contact with a female fuses to her body and reduces most organ systems other than the reproductive organs (Pietsch, 2005). Even in its pre-fusion, free-swimming stage, these male anglerfish are among the smallest vertebrates (Table 1). These fish are anatomically but not ecologically parasites, since they do not reduce the fitness of their female mates to which they are attached to. This interesting example is restricted to one life-history stage of one sex of a few species.

Several miniaturized vertebrate species show paedomorphism, the early truncation of development, and *Paedocypris* and *Paedophryne* even carry the syllable *Paedo*- in their names. Even though it is believed to have played roles in the origin of several major vertebrate lineages (Pérez-Ben et al., 2017), paedomorphism is seemingly rare in vertebrates, and does not find extreme expressions.

Overall the picture emerges that a reduction of bodily complexity is achievable only to a moderate degree in vertebrates and might have limited the evolutionary development of extremely small vertebrate species.

Indirect Limit on Adult Body Size due to Minimum Number of Necessary Eggs

So far, we have argued that the complex Bauplan of vertebrate bodies, and especially vertebrate brains sets a lower size limits for vertebrate bodies. We now extend the argument to take life-history into account. The minimum body size obtainable by vertebrates outlined above will set a lower limit to the size of the smaller larvae and juveniles rather than the larger adult animals. In turn, to produce a behaviorally functioning juvenile or larva above a minimum size, vertebrate eggs will need to be above a certain size. This lower limit on egg size will limit the number of eggs per female as adult females evolutionarily decrease in size.

The population replacement value is the number of offspring per female which is needed to keep the population at a constant value. The fecundity needs to replace losses due to disease, parasitism and predation. If the number of offspring per female drops below the replacement value, then the situation is not evolutionarily stable and the population and eventually the species will go extinct. When a species evolved towards smaller and smaller sizes, at one point the possible number of eggs per female will reach the replacement value, making a further size reduction impossible. We demonstrate this relationship between body size and life-time fecundity in gobiid fishes (Herler et al, 2011), a well-researched group in terms of their life-histories (Fig. 3, Tab. 2).

Max size (mm)	**Life time fecundity**	**Species**	**Reference**
8.7	54	*Trimmatom nanus*	Winterbottom, & Emery, 1981
18	243	*Eviota sigillata*	Depczynski & Bellwood, 2006
24	800	*Knipowitschia mermere*	Özcan, G., 2009
25.7	1,039	*E. queenslandica*	Depczynski & Bellwood, 2006
27	270	*Lebetus scorpioides*	Miller, P.J., 1986
27.1	781	*Eviota melasma*	Depczynski & Bellwood, 2006
39.5	2,453	*Pomatoschistus lozanoi*	Claridge et al. 1985
45	2,648	*Aphia minuta*	Iglesias & Morales-Nin, 2001
48	1,226	*Pomatoschistus marmoratus*	Miller, 1986
50	5,603	*Pomatoschistus minutus*	Claridge et al. 1985
64	2,000	*Clevelandia ios*	Hart, 1973
66	46,000	*Sicyopterus lagocephalus*	Manacop, 1953
80	2,100	*Periophthalmus barbarus*	Turay et al. 2006
85	2,500	*Benthophilus stellatus*	Miller, 1986
88	8,978	*Gobius paganellus*	Miller, 1986
105	224,960	*Sicyopterus japonicus*	Miller, 1984
110	2,888	*Neogobius fluviatilis*	Troitsky & Tsunikova, 1983
121	38,443	*Amblygobius phalaena*	Takegaki, 2000
129	164,633	*Valenciennea strigata*	Reavis, 1997
150	5,000	*Neogobius melanostomus*	Skora et al., 1999
162	2,236	*Babka gymnotrachelus*	Grabowska, 2005
166	3,824	*Neogobius melanostomus*	Tomczak & Sapota, 2006
187	2,190	*Neogobius melanostomus*	Tomczak & Sapota, 2006
240	335,034	*Glossogobius giuris*	Machacek (ed.), 2010
250	1,818	*Neogobius melanostomus*	McInnis & Corkum, 2000

Table 2: Maximum adult size and life-time fecundity of gobies (Gobiidae), including references for the data points. See Fig. 3 for a graphic display of this dataset. Note that the methods for determining fecundity were not identical between studies, however our argument is based on order-of-magnitude estimates of fecundity, not on precise values.

When observing the fecundity-length relationship in gobies, we see two species at the small end of the range: In *Eviota stigillata* (TL 18 mm) females produce about 243 eggs per lifetime (Depczynski and Bellwood, 2005; 2006), and in *Trimmatom nanus* (TL 8.7 mm) the life-time fecundity is only 54 (Winterbottom, & Emery, 1981). These values are possibly near the minimum possible for minute (highly preyed upon) vertebrate species. An aggravating factor is that smaller animals typically are lower in the trophic chain (and are what was formerly termed “r-breeders”) and likely require a higher number of eggs per female to reach population replacement value. Hence, this second part of our argument is indirect and argues that life-history and ecological factors *further* limit the minimum attainable body size of vertebrates.

In this context it is worth noting that some of the smallest frogs are direct developers, without a free-living tadpole stage (Callery et al. 2001). Direct developing frog species have smaller adult size and tend to have large eggs (Callery et al. 2001). Large egg size among direct developing frogs has been found correlated with parental care (Summers et al., 2007). Cryptobenthic reef fishes, the smallest part of the vertebrate fauna in coral reefs, often employ reproductive behaviors which increase offspring survival, such as mouth-brooding in cardinalfishes or egg-guarding in gobies(Brandl et al., 2018).

Also, in many miniaturized species, females are larger than males, such as in the goby *Schindleria* (Watson & Walker, 2004), again pointing to female body size as the limiting factor for small body sizes. The parasitic males of deep-sea anglerfish (*L. pusillus*) are of course an extreme example of this size disparity between the sexes of miniaturized species (Roberts, 2013).

The difference to invertebrates is that much smaller limit on egg size will allow even very small females to produce a number of eggs above the replacement value needed for the animal’s ecological situation. This argument rests on a positive relationship between egg size and the size of the larva or juvenile (Emlet & Hoegh-Guldberg, 1997). It has to be pointed out that the relationship between organism size and egg size is not strictly linear, and incompletely understood. While these findings should be kept in mind, it’s still a reasonable assumption that larger eggs will generally allow the development of larger larvae or juveniles.

Hypothesis	**Supporting arguments**	**Counter-arguments**
Organ complexity, especially of the brain, is limiting smaller body sizes.	Vertebrate bodies contain more cell types and more complex organs, especially the nervous system. The cell type number is higher.	Some paedomorphic and parasitic vertebrates exist with reduced (albeit not radically reduced) Baupläne

Eggs per female drop below replacement number with small body sizes.	Small animals experience high mortality due to predation, which aggravate the effect.	The relationship between egg size and development is complex and poorly understood.

Table 3: Possible explanations for the lower size limits on vertebrates and the arguments supporting and opposing them.

Discussion

We have outlined a hypotheses which could explain the observation that the minimum size of adult vertebrates does not lie below 6 millimeters in any species, primarily based on a minimum brain size necessary to maintain brain function. Adding to this, constrains stemming from life history and ecology do not allow egg number to drop below certain limits, hence limiting adult female size (Fig. 4).

Few previous explanations for the lower size limit of vertebrates have been proposed. Notably, a limit for the settlement size of reef fishes has been shown to exist for tropical coral reef fishes, caused by parasitic pressure (Grutter et al., 2017). Fishes below this settlement size are too weakened by parasites to survive. The question this raises is why parasitism does not impose a size limit on other phyla? Likely, rather than parasites limiting the minimum size of reef fishes, parasites evolved to prey on newly settling larvae of sizes limited by the factors discussed above.

It is also noteworthy that the minimum size for cephalopods is in a similar range to the minimum size of vertebrates (Shigeno et al., 2010). The smallest known cephalopod is *Idiosepius notoides*, with mature males measuring as little as 6 mm (Tracey et al., 2003, Fig. 2). Cephalopods are a group of animals with a similarly complex Bauplan, with many species using large brains and well-developed sensory- and locomotor systems for an active, hunting life-style. Similarly to vertebrates, a reduction of the complex cephalopod Bauplan might be incompatible with their niches which depend on complex behavior. Cephalopods have been called “honorary vertebrates” due to their unusually complex behavior and large brains (Shigeno et al., 2018). The anatomy of the cephalopod brain with its multiple cortices and lobes is more reminiscent of vertebrates than of its distant mollusk relatives such as the bivalves and gastropods. It is tempting to speculate that the minimum size limits in vertebrates and cephalopods had evolved convergently, in both cases based on constraints imposed by a lower limit of brain size (see also Martin, 1981).

Miniaturization is even more challenging for mammals, which maintain a constant body temperature above the ambient temperature. This heterothermy makes the unfavorable scaling of energy use even more unfavorable; their larger brains make the unfavorable scaling of brain size with increasingly smaller sizes even more unfavorable. Hence it is not a surprise that the smallest mammals are significantly larger than the smallest non-mammalian amniotes. The Etruskan shrew (*Suncus etruscus*, 40 mm) and the bumblebee bat (*Craseonycteris thonglongyai*, 29 mm) are considered the smallest mammalian species.

Interestingly, there has been a considerable amount on neurobiological research done on the Etruskan shrew. The conclusions are that the Etruskan shrew is an extreme tactile specialist, which is reflected in the large proportion of its cerebral cortex dedicated to tactile representation. The cortical maps of the Etruskan shrew look fundamentally similar to those of larger shrew species (Roth-Alpermann et al., 2010; Naumann et al., 2012). Brecht et al., (2011) argue that "high-speed behavior and extreme dependence on touch are not coincidental, but reflect an evolutionary strategy, in which the metabolic costs of small body size are outweighed by the advantages of being a short-range high-speed touch and kill predator". Ray et al., (2020) showed that the Etruskan shrew's brain, especially their neocortex, shrinks in winter, when its body temperature is also reduced. Such drastic adaptations specific to the brain are in accord with an important role of the brain's energy use in limiting the size of minute species.

Evolutionary Context

While large body sizes are advantageous for animals in many ways ("Cope's rule"; Hone & Benton 2005; Stiefel, 2021), small species are more likely to arise due to the larger number of individuals in populations of small-bodied species (Stiefel & Quimpo, 2017). The pool of candidate small species is hence large in which further miniaturization can occur, to fill a variety of niches ideally fit for small body sizes. This is also the case for vertebrates, for instance in bony fishes the body mass of 53% of recorded species is below 126 g (Stiefel & Quimpo, 2017). The absence of extremely miniaturized species in vertebrates is hence not due to a lack of small bodies-species, but rather most likely due to barriers related to vertebrate Bauplan and life-histories. We believe we have outlined a combination of such barriers which provides a reasonable explanation for the difference of these barriers in vertebrates and invertebrates.

Acknowledgments

We would like to thank Dr. Celeste Pérez Ben for valuable discussion of the topics covered in this study.

References

Biju, S. D., Van Bocxlaer, I., Giri, V. B., Roelants, K., Nagaraju, J., & Bossuyt, F. (2007). A new nightfrog, *Nyctibatrachus minimus* sp. nov. (Anura: Nyctibatrachidae): the smallest frog from India. *Current Science*, 854-858.

Bieler, R., & Mikkelsen, P. M. (1998). Ammonicera in Florida: Notes on the smallest living gastropod in the United States and comments on other species of Omalogyridae (Heterobranchia). *Nautilus-Paper Edition*, 111(1), 1-12.

Brandl, S. J., Goatley, C. H., Bellwood, D. R., & Tornabene, L. (2018). The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs. *Biological Reviews*, 93(4), 1846-1873.

Brecht, M., Naumann, R., Anjum, F., Wolfe, J., Munz, M., Mende, C., & Roth-Alpermann, C. (2011). The neurobiology of Etruscan shrew active touch. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1581), 3026-3036.

Buzsáki, G., Geisler, C., Henze, D. A., & Wang, X. J. (2004). Interneuron diversity series: circuit complexity and axon wiring economy of cortical interneurons. *Trends in Neurosciences*, 27(4), 186-193.

Callery, E. M., Fang, H., & Elinson, R. P. (2001). Frogs without polliwogs: evolution of anuran direct development. *BioEssays*, *23*(3), 233-241.

Claridge, P. N., Hardisty, M. W., Potter, I. C., & Williams, C. V. (1985). Abundance, life history and ligulosis in the gobies (Teleostei) of the inner Severn estuary. *Journal of the Marine Biological Association of the United Kingdom*, 65(4), 951-968.

DeFelipe, J., López-Cruz, P. L., Benavides-Piccione, R., Bielza, C., Larrañaga, P., Anderson, S., ... & Ascoli, G. A. (2013). New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nature Reviews Neuroscience*, 14(3), 202-216.

Depczynski, M., & Bellwood, D. R. (2006). Extremes, plasticity, and invariance in vertebrate life history traits: insights from reef fishes. *Ecology, 87*(12), 3119–3127.

Dwyer, S. L., & Visser, I. N. (2011). Cookie cutter shark (*Isistius* sp.) bites on cetaceans, with particular reference to killer whales (orca)(*Orcinus orca*). *Aquatic Mammals, 37*(2), 111-138.

Eberhard, W. G. (2007). Miniaturized orb-weaving spiders: behavioural precision is not limited by small size. *Proceedings of the Royal Society B: Biological Sciences*, 274(1622), 2203-2209.

Emlet, R. B., & Hoegh‐Guldberg, O. (1997). Effects of egg size on postlarval performance: experimental evidence from a sea urchin. *Evolution*, *51*(1), 141-152.

Froese, R., Thorson, J. T., & Reyes Jr, R. B. (2014). A Bayesian approach for estimating length-weight relationships in fishes. *Journal of Applied Ichthyology*, *30*(1), 78-85.

Grabowska, J. (2005). Reproductive biology of racer goby *Neogobius gymnotrachelus* in the Włocławski Reservoir (Vistula River, Poland). *Journal of Applied Ichthyology*, 21(4), 296-299.

Grutter, A. S., Blomberg, S. P., Fargher, B., Kuris, A. M., McCormick, M. I., & Warner, R. R. (2017). Size-related mortality due to gnathiid isopod micropredation correlates with settlement size in coral reef fishes. *Coral Reefs*, *36*(2), 549-559.

Hedges, S. B., & Thomas, R. (2001). At the lower size limit in amniote vertebrates: a new diminutive lizard from the West Indies. *Caribbean Journal of Science*, *37*(3/4), 168-173.

Hart, J.L. (1973). Pacific Fishes of Canada. *Bulletin Fisheries Research Board Canada* No. 180, 740 pp.

Hedges, S. B., & Thomas, R. (2001). At the lower size limit in amniote vertebrates: a new diminutive lizard from the West Indies. *Caribbean Journal of Science*, 37(3/4), 168-173.

Herculano-Houzel, S., & Lent, R. (2005). Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. *Journal of Neuroscience*, 25(10), 2518-2521.

Herler, J., Munday, P. L., & Hernaman, V. (2011). Gobies on coral reefs. *The Biology of Gobies*, 493-529.

Herre, A. W. (1929). The smallest living vertebrate. *Science*, *69*(1786), 329-330.

Hone, D. W., & Benton, M. J. (2005). The evolution of large size: how does Cope's Rule work?. *Trends in Ecology & Evolution*, 20(1), 4-6.

Iglesias, M., & Morales-Nin, B. (2001). Life cycle of the pelagic goby *Aphia minuta* (Pisces: Gobiidae). *Scientia Marina*, 65(3), 183-192.

Kaur, H., Attri, R., & Joshi, J. (2016). Molecular identification of a new myxozoan, *Myxobolus dermiscalis* n. sp.(Myxosporea) infecting scales of *Labeo rohita* Hamilton in Harike Wetland, Punjab (India). *International Journal for Parasitology: Parasites and Wildlife*, *5*(2), 139-144.

Kottelat, M., Britz, R., Hui, T. H., & Witte, K. E. (2006). Paedocypris, a new genus of Southeast Asian cyprinid fish with a remarkable sexual dimorphism, comprises the world's smallest vertebrate. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1589), 895-899.

Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brännström, I., ... & Kolm, N. (2013). Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Current Biology*, 23(2), 168-171.

Liao, W. B., Lou, S. L., Zeng, Y., & Kotrschal, A. (2016). Large brains, small guts: the expensive tissue hypothesis supported within anurans. *The American Naturalist*, 188(6), 693-700.

Machacek, H. (ed.), (2010). World Records Freshwater Fishing. www.fishing-worldrecords.com, September 2010.

Manacop, P. R. (1953). The life history and habits of the goby, *Sicyopterus extraneus* Herre (anga) Gobiidae with an account of the goby-fry fishery of Cagayan River, Oriental Misamis. *Philippine Journal of Fisheries*, 2, 1-57.

Martin, R. D. (1981). Relative brain size and basal metabolic rate in terrestrial vertebrates. *Nature*, 293(5827), 57-60.

Martin, J. W., & Davis, G. E. (2001). *An updated classification of the recent Crustacea* (Vol. 39, p. 129). Los Angeles: Natural History Museum of Los Angeles County.

McShea, D. W. (1992). A metric for the study of evolutionary trends in the complexity of serial structures. *Biological Journal of the Linnean Society*, 45(1), 39-55.

MacInnis, A. J., & Corkum, L. D. (2000). Fecundity and reproductive season of the round goby *Neogobius melanostomus* in the upper Detroit River. *Transactions of the American Fisheries Society*, 129(1), 136-144.

Miller, P. J. (1986). Gobiidae. Fishes of the North-eastern Atlantic and Mediterranean, 3, 1019-1085.

Mockford, E. L. (1997). A new species of Dicopomorpha (Hymenoptera: Mymaridae) with diminutive, apterous males. *Annals of the Entomological Society of America*, *90*(2), 115-120.

Naumann, R. K., Anjum, F., Roth-Alpermann, C., & Brecht, M. (2012). Cytoarchitecture, areas, and neuron numbers of the Etruscan shrew cortex. *Journal of Comparative Neurology*, 520(11), 2512-2530.

Özcan, G. (2009). The status of the Freshwater Gobiid, *Knipowitschia mermere* Ahnelt, 1995: distribution ecology and threats. *Biharean Biologist*, 3(2), 139-141.

Pérez-Ben, C. M., Schoch, R. R., & Báez, A. M. (2018). Miniaturization and morphological evolution in Paleozoic relatives of living amphibians: a quantitative approach. *Paleobiology*, *44*(1), 58–75.

Pietsch, T. W. (2005). Dimorphism, parasitism, and sex revisited: modes of reproduction among deep-sea ceratioid anglerfishes (Teleostei: Lophiiformes). *Ichthyological Research*, 52(3), 207-236.

Reavis, R. H. (1997). The natural history of a monogamous coral-reef fish, *Valenciennea strigata* (Gobiidae): 2. behavior, mate fidelity and reproductive success. *Environmental Biology of Fishes*, 49(2), 247-257.

Rittmeyer, E. N., Allison, A., Gründler, M. C., Thompson, D. K., & Austin, C. C. (2012). Ecological guild evolution and the discovery of the world's smallest vertebrate. *PLoS one*, *7*(1), e29797.Ray, S., Li, M., Koch, S. P., Mueller, S., Boehm-Sturm, P., Wang, H., ... & Naumann, R. K. (2020). Seasonal plasticity in the adult somatosensory cortex. *Proceedings of the National Academy of Sciences*, 117(50), 32136-32144.

Roberts, T. R. (2013). Leptophilypnion, a new genus with two new species of tiny central Amazonian gobioid fishes (Teleostei, Eleotridae). *Aqua, International Journal of Ichthyology*, 19(2-26), 85-98.

Roth-Alpermann, C., Anjum, F., Naumann, R., & Brecht, M. (2010). Cortical organization in the Etruscan shrew (*Suncus etruscus*). *Journal of Neurophysiology*, 104(5), 2389-2406.

Sato, T. (1986). A brood parasitic catfish of mouthbrooding cichlid fishes in Lake Tanganyika. *Nature*, *323*(6083), 58.

Shigeno, S., Takenori, S., & Boletzky, S. V. (2010). The origins of cephalopod body plans: a geometrical and developmental basis for the evolution of vertebrate-like organ systems. *Cephalopods-Present and Past*, *1*, 23-34.

Shigeno, S., Andrews, P. L., Ponte, G., & Fiorito, G. (2018). Cephalopod brains: an overview of current knowledge to facilitate comparison with vertebrates. *Frontiers in Physiology*, *9*, 952.

Skora, K., Olenin, S & Gollasch, S (1999) *Neogobius melanostomus* (Pallas, 1811). In: Gollasch S, Minchin D, Rosenthal H, Voigt M (eds) Case histories on introduced species: th eir general biology, distribution, range expansion and im pact, pp. 69-73, Logos-V erlag, Berlin.

Stiefel, K. M., & Quimpo, T. J. R. (2017). Fish Diversity as a Function of Depth and Body Size. *BioRxiv*, 208397.

Stiefel, K. M. (2021). Evolutionary trends in large pelagic filter-feeders. *Historical Biology*, 33(9), 1477-1488.

Stopfer, M., Jayaraman, V., & Laurent, G. (2003). Intensity versus identity coding in an olfactory system. *Neuron*, 39(6), 991-1004.

Striedter, G. F. (2005). Principles of brain evolution. Sinauer associates, Massachusetts, USA.

Summers, K., McKeon, C. S., Heying, H., Hall, J., & Patrick, W. (2007). Social and environmental influences on egg size evolution in frogs. *Journal of Zoology*, *271*(2), 225-232.

Sompolinsky, H., Yoon, H., Kang, K., & Shamir, M. (2001). Population coding in neuronal systems with correlated noise. *Physical Review E - Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics*, *64*(5), 11.

Takegaki, T. (2000). Monogamous mating system and spawning cycle in the gobiid fish, *Amblygobius phalaena* (Gobiidae). *Environmental Biology of Fishes*, 59(1), 61-67.

Taucce, P. P., Costa-Campos, C. E., Haddad, C. F., & de Carvalho, T. R. (2020). A New Amazonian Species of the Diminutive Frog Genus *Adelophryne* (Anura: Brachycephaloidea: Eleutherodactylidae) from the State of Amapá, Northern Brazil. *Copeia*, *108*(4), 746-757.

Tenaillon, O., Silander, O. K., Uzan, J. P., & Chao, L. (2007). Quantifying organismal complexity using a population genetic approach. *PloS one*, 2(2), e217.

Thompson, D. K., Austin, C. C., Rittmeyer, E. N., Allison, A., & Gru, M. C. (2012). Ecological Guild Evolution and the Discovery of the World ' s Smallest Vertebrate, *7*(1), 1–11.

Tomczak, M. T., & Sapota, M. R. (2006). The fecundity and gonad development cycle of the round goby (*Neogobius melanostomus* Pallas 1811) from the Gulf of Gdańsk. *Oceanological and Hydrobiological Studies*, 35(4), 353-367.

Tracey, S. R., Steer, M. A., & Pecl, G. T. (2003). Life history traits of the temperate mini-maximalist Idiosepius notoides,(Cephalopoda: Sepioidea). *Journal of the Marine Biological Association of the United Kingdom*, *83*(6), 1297-1300.

Troitsky, S.K. & Tsunikova, E.P. (1983). Biology of monkey goby *Neogobius fluviatilis* (Pallas) (Gobiidae) in estuaries of Kuban river. *Vorposy Ikhtiologii* 23(4), 569-574.

Turay, I., Vakily, J. M., Palomares, M. L. D., & Pauly, D. (2006). *Periophthalmus barbarus* on mudflats of Freetown, Sierra Leone. *Fisheries Centre Research Reports*, 14(4), 49.

Watson, W. I. L. L. I. A. M., & Walker, H. J. (2004). The world's smallest vertebrate, *Schindleria brevipinguis*, a new paedomorphic species in the family Schindleriidae (Perciformes: Gobioidei). *Records-Australian Museum*, *56*(2), 139-142.

Willmore, K. E. (2012). The body plan concept and its centrality in evo-devo. *Evolution: Education and Outreach*, *5*(2), 219-230.

Winterbottom, R. (1990). The *Trimmatom nanus* species complex (Actinopterygii, Gobiidae): phylogeny and progenetic heterochrony. *Systematic Zoology*, *39*(3), 253-265.

Winterbottom, R., & Emery, A. R. (1981). *Trimmatom nanus* new genus new species and *Trimmatom offucius* new species of gobiid fishes (Perciformes) from the Chagos Archipelago, Central Indian Ocean. *Environmental Biology of Fishes*, 6, 139-150.

Valentine, J. W., Collins, A. G., & Meyer, C. P. (1994). Morphological complexity increase in metazoans. *Paleobiology, 20*(2), 131-142.

Figures

Figure 1: Examples of exceptionally small vertebrate species.

Paedophryne amauensis, frog, 7.7 mm, photo from Thompson et al., 2012.

Eviota sigillata, gobiidae, bony fish, <10 mm, photograph by K.M.S.

Schindleria pietschmanni, and *S. praematura*, gobiidae, teleosts (bony fish), 15/20 mm, drawing from Johnson & Brothers, 1993.

Images not to the same scale.

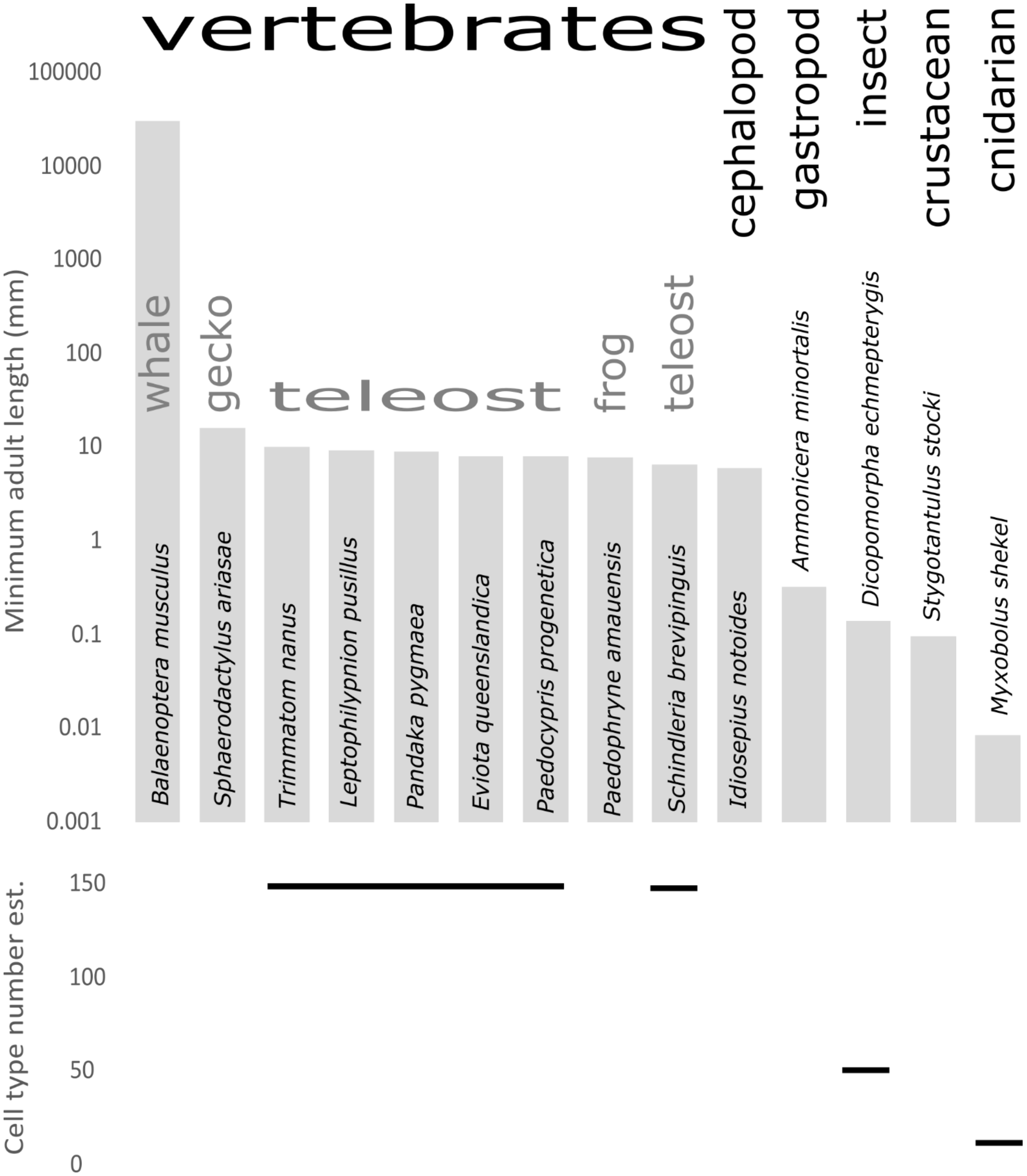

Figure 2: Adult body lengths (top, see Tab. 1) for a selection of miniaturized vertebrate (left) invertebrate species (right) and the estimated number of cell types according to Valentine, 1994 (bottom) for different phyla. Blue whale (*Balaenoptera musculus*) included for comparison.

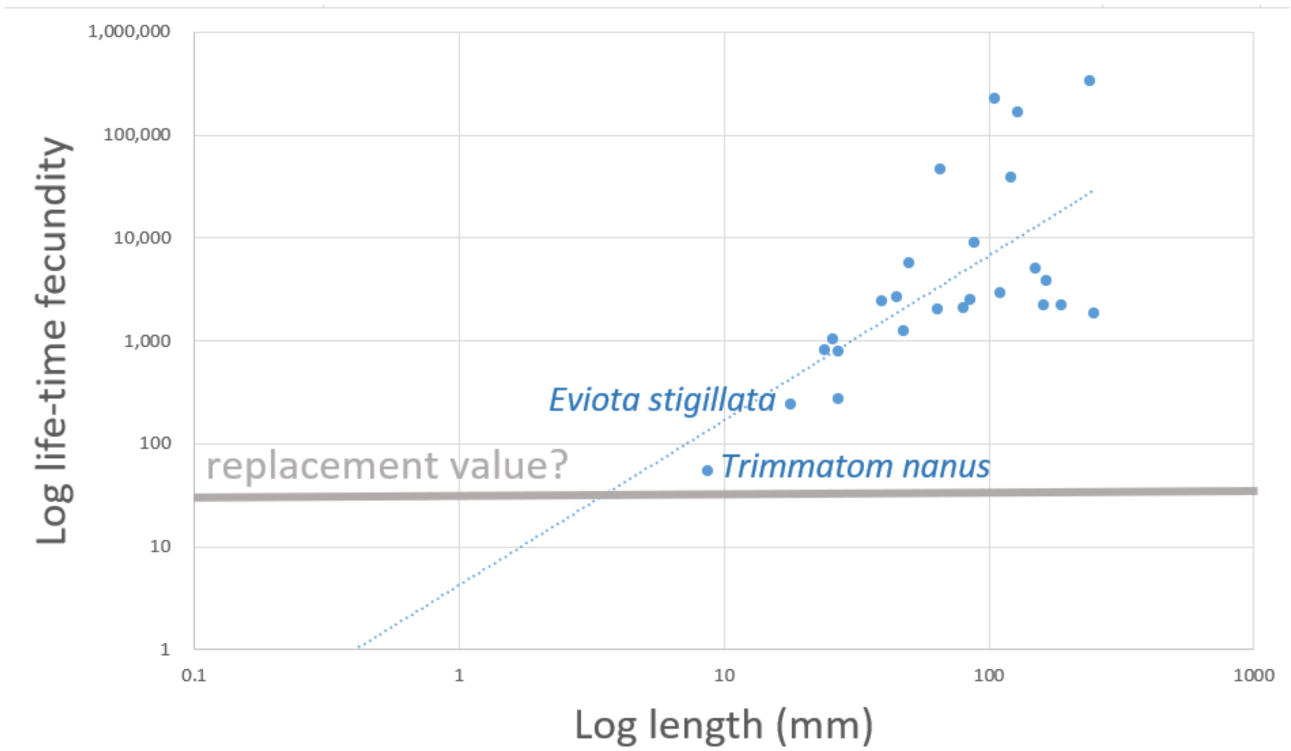

Figure 3: Relationship between fecundity per female and body size in gobies (Gobiidae). Data are from the sources cited in Table 2. The dashed blue line is the power-function regression of the log/log plot. Our hypothesis is that a fecundity level exists where a further reduction of body size will push the fecundity below population replacement value.

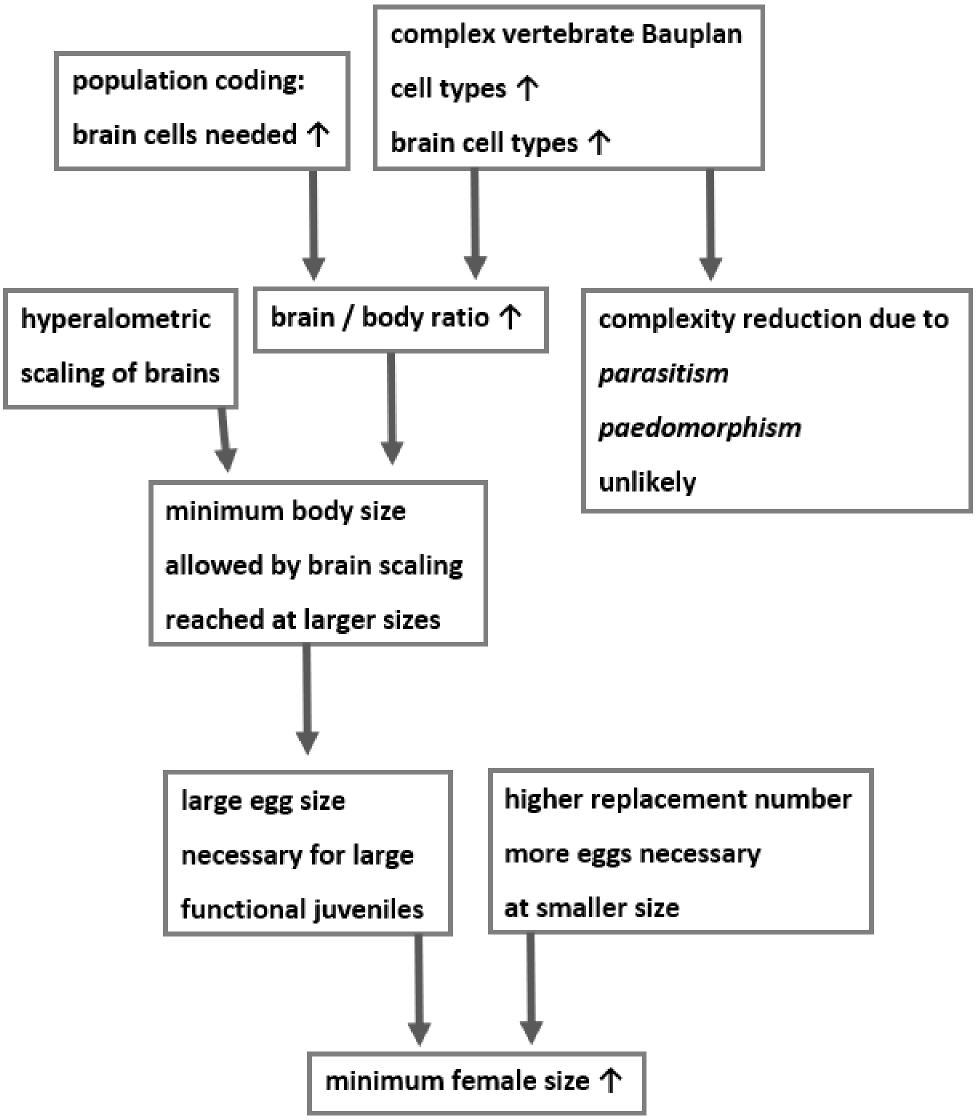

Figure 4: Schematic outline of the argument presented in the article. The complex brains of vertebrates employ population coding, which makes the reduction of the neurons in several brain centers not feasible. As a consequence, the miniaturization of vertebrate brains and bodies is restricted. This affects smaller juvenile and larval animals more than adults, and sets a minimum egg size. This minimum egg size, together with a minimum number of eggs necessary to reach the population replacement value.