

COMMENTARY

The good, the bad and the slimy: experimental studies of hagfish digestive and nutritional physiology

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ABSTRACT

The hagfishes provide valuable insight into the physiology of feeding, digestion and nutrient absorption by virtue of unusual and unique features of their biology. For example, members of this group undergo long periods of fasting, and are the only vertebrates known to absorb organic nutrients across their epidermal surface. Such properties engender significant attention from researchers interested in feeding and feeding-related processes; however, the practical realities of employing the hagfish as an experimental organism can be challenging. Many of the key tools of the experimental biologist are compromised by a species that does not readily feed in captivity, is difficult to instrument and which produces copious quantities of slime. This Commentary provides critical insight into the key aspects of hagfish feeding and digestive processes, and highlights the pitfalls of this group as experimental organisms. We also suggest key research gaps that, if filled, will lead to better understanding of hagfishes, and we consider how this group may advance our knowledge of feeding, digestion and nutrient absorption processes.

KEY WORDS: Digestion, Evolution, Feeding, Hagfish, Nutrition, Slime

Introduction

Every aspect of an animal's biology is shaped either directly or indirectly by the processes of feeding, digestion and nutrient assimilation. Ecological niche, intra- and inter-species interactions, behaviour, physiology, biochemistry and molecular function are all influenced by the need for, or consequences of, the successful acquisition of nutrients (Karasov et al., 2011). Developing an understanding of how an organism acquires food, breaks this food down into absorbable nutrients and then assimilates these compounds is therefore critical to a wide range of biological disciplines. A key tool that facilitates this insight is the use of species which, by virtue of the characteristics of their feeding biology and/or their phylogenetic placement, offer insight into the functions, mechanisms or evolution of digestive and absorptive processes. These species are particularly valuable when coupled with experimental biology methods that manipulate the environmental and physiological state of the animal. Such approaches provide an understanding of how feeding and feeding-related processes are regulated, and how these processes ultimately contribute to organism homeostasis.

Hagfishes are one vertebrate group with huge potential for advancing our understanding of feeding, digestion and nutrition.

There are currently 82 characterised hagfish species (FishBase, <https://www.fishbase.se/>; April, 2019), with the hagfish lineage having diverged from the main line of vertebrate evolution approximately 560 million years ago (Kumar and Hedges, 1998). The specific phylogenetic placement of this group is subject to frequent re-examination and revision (see Box 1), leading to discussion as to whether the traits of hagfish are primitive (i.e. representative of the earliest vertebrates) or derived (i.e. representative of an animal adapted to its benthic marine habitat). This is an important distinction, as it dictates the value of hagfishes as species capable of providing evolutionary insight. However, such conclusions are usually only able to be drawn by comparative analysis of a given trait in hagfishes, other chordates and advanced invertebrates (e.g. Nakashima et al., 2018). To date, very few such studies exist. Nevertheless, there is significant value in studying hagfish biology in an evolutionary context, even if only to establish fundamental knowledge that can eventually be incorporated into detailed cross-species analyses.

Among the unusual traits of hagfish that make them of particular interest from the perspective of digestive and nutritional physiology are their long periods of fasting and their utilisation of multiple epithelia for the acquisition of nutrients. Hagfishes are principally characterised as opportunistic scavengers, relying on the presence of decaying carrion that settles on the seafloor. The poor availability of such feeding opportunities may necessitate long periods of fasting. Indeed, in captivity, hagfish can survive for at least 11 months without feeding (Foster and Moon, 1986). By analogy with other intermittent feeders (see Glossary), periods of feast and famine may require significant physiological plasticity. For example, in the intermittently feeding pythons, physiological plasticity extends from the structures and functions of the gut itself to the changes required in other physiological systems in order to withstand extended phases without energy and nutrient inputs (Secor, 2008). Therefore, studies of digestive and nutrient functions in species such as hagfishes may provide insight into endocrine control of satiation, tissue-remodelling processes, strategies for energy conservation and the underlying mechanisms that drive these phenomena.

When scavenging opportunities are available, hagfish display some curious feeding behaviours. Upon encountering seafloor carrion, a hagfish will bore a hole through the carcass and burrow into the cavity to preferentially access soft tissues (Martini, 1998). This immersive feeding (see Glossary) is proposed to expose the skin and gills to an enriched milieu of decaying organic nutrients, which may be the driver for an unusual characteristic: integumental organic nutrient absorption (Stephens, 1968; Glover et al., 2011a). The use of the skin and gills, in addition to the gut, for nutrient absorption offers an intriguing system in which to study epithelial crosstalk, the physiological compromises of multiple multi-functional epithelia, and the adaptive value of maximising nutritional value from a sporadic food source.

In this Commentary, we discuss recent research that has started to characterise the feeding-related physiology of hagfishes. We then

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Glossary

Alkaline tide. A phenomenon occurring after feeding whereby blood pH is elevated by bicarbonate ions released from the proton-producing gut cells involved in acidic digestion.

Blood sinus system. An interconnected system of blood spaces in hagfish, akin to the secondary vascular system of teleost fish.

Gavage. The administration of food by force, usually via introduction of a liquefied slurry directly into the gastrointestinal tract.

Immersive feeding. A feeding mode involving immersion in the food source.

Intermittent feeding. A feeding mode whereby meals are infrequent.

Osmoconformer. An animal that has an extracellular fluid composition that matches the osmolality of its environment.

Osmoregulator. An animal that regulates its extracellular fluid composition, so that its osmolality may differ from that of its environment.

Regular feeding. A feeding mode whereby meals are frequently consumed.

Specific dynamic action (SDA). The costs of digestion and meal assimilation, usually determined by an increase in metabolic rate/oxygen consumption.

detail some of the practical pitfalls of working with this group, including the complicating factor of their notable slime production. Finally, we address some of the key remaining knowledge gaps that will help to contextualise much of the current data and shape future research on this fascinating group.

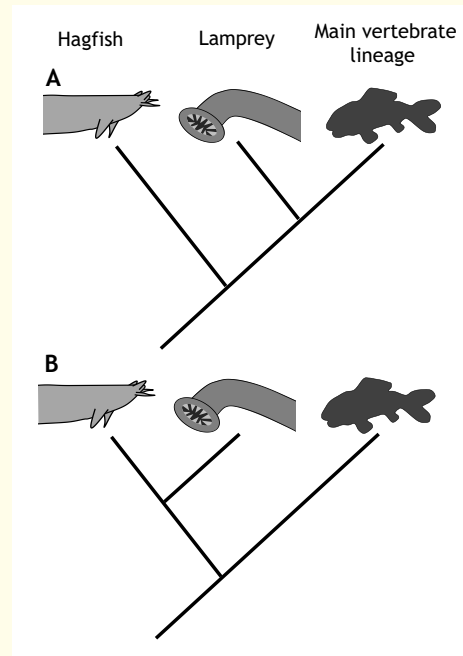
The good: intriguing aspects of hagfish digestive and nutritive physiology

An unusual feeding niche

By burrowing into decaying seafloor carcasses, hagfishes are able to use their gill and skin epithelia to absorb amino acids directly from the water (Stephens, 1968; Glover et al., 2011a). This ability is well described among invertebrates (e.g. Gomme, 2001), but is unique to hagfish within vertebrates. Research to date has shown that uptake is saturable (i.e. not diffusive; Glover et al., 2011a), indicative of mediation via amino acid transporters. As yet, the particular transporters responsible have not been structurally characterised, nor have the cell types that achieve transport been identified. Absorptive pathways across the skin and gill are specific (i.e. capable of being inhibited by putative transport competitors), and regulated by factors such as nutritive state and the presence of environmental hypoxia (Bucking et al., 2011; Glover et al., 2011a, 2016). The relative importance of the different epithelia to overall nutrient uptake is difficult to assess, owing to the differences in ambient nutrient concentrations associated with each epithelium, and the fact that total epithelial surface areas have not been determined in studies to date. However, on a per surface area basis, maximal transport rates of the amino acid alanine across the gut are approximately 3.8-fold greater than those across the skin (Glover et al., 2011a,b). Relative nutrient uptake affinities show a much higher Michaelis–Menten affinity constant (i.e. lower uptake affinity) for amino acids in the gut of hagfish ($\sim 2\text{--}7\text{ mmol l}^{-1}$) compared with both the skin and gill ($\sim 125\text{--}465\text{ }\mu\text{mol l}^{-1}$; Glover and Bucking, 2016). These differences define the transport pathways of the skin and gill as high-affinity, low-capacity uptake pathways, relative to the low-affinity, high-capacity systems of the gut. This reflects the relative concentrations of amino acids likely to be encountered at each surface (i.e. higher nutrient contents in food than in water or sediment). Consequently, while the gut performs the bulk of the amino acid transport, the skin and gill may have critical supplementary roles, in particular when the animal is fasting (see

Box 1. Phylogeny and the utility of hagfishes as model species

The value of hagfishes as evolutionary models depends in part on their phylogenetic placement. One theory (A in figure), largely supported by morphological data (e.g. Forey and Janvier, 1993), contends that lampreys and hagfishes split independently from the main vertebrate lineage, and therefore their shared primitive characteristics are likely representative of the earliest vertebrates. The second theory (B in figure), which is supported by molecular analysis (e.g. Heimberg et al., 2010) and a recent discovery of an ancient fossil hagfish (Miyashita et al., 2019) suggests that hagfish and lamprey share a common ancestor; the primitive features of these animals may be a consequence of adaptation within this radiation, and as such they may be quite distinct from the earliest vertebrates. Consequently, the utility of hagfishes as an evolutionary model species for understanding basal vertebrate traits is greater under the former hypothesis than the latter.



below). It is also important to note that use of the skin as an uptake surface is not restricted to organic nutrients, but inorganic elements (e.g. phosphorus, nickel, iron) may also be absorbed across this surface via specific uptake pathways (Schultz et al., 2014; Glover et al., 2015, 2016). It remains to be determined whether the transport capacity of the integument extends to carbohydrate and lipid nutrients.

It has been proposed that the capacity of the skin to take up nutrients directly from the water is a consequence not only of the enriched dissolved nutrient concentrations of the immersive feeding environment, but also a function of the hagfishes' osmoconforming strategy (see Glossary; Fig. 1; Bellamy and Chester-Jones, 1961). As the extracellular fluid osmolality of hagfish matches that of their environment, net ion and water fluxes are minimised. This reduces the need to utilise the integument as a barrier, leading to an epidermal structure that favours exchange over protection, and ultimately results in a permeable skin surface capable of acquiring nutrients (Weinrauch et al., 2016; Glover et al., 2017). By contrast, osmoregulating animals (see Glossary) rely on minimal exchange of ions and water across their epidermal epithelia to reduce the costs of regulation. Two key factors facilitate this reduced environmental exchange in later-diverging vertebrates: an impermeable integument

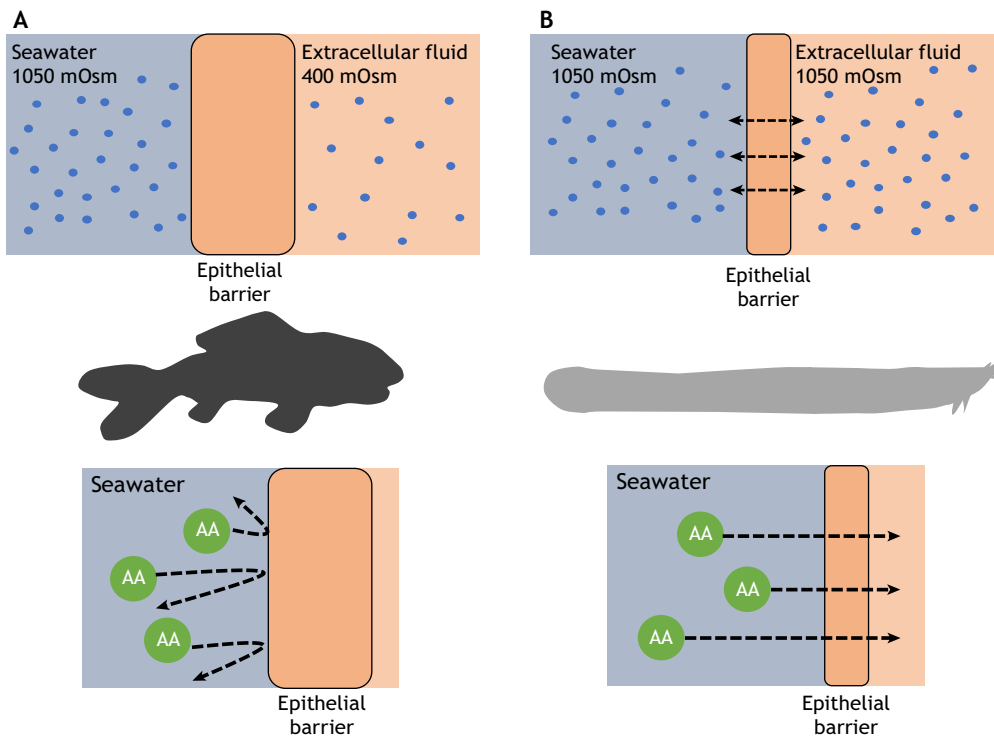


Fig. 1. Osmoregulatory strategy, epidermal permeability and capacity to use the skin as an absorptive surface. (A) In an osmoregulating vertebrate, the skin epithelium is impermeable to salt and water movement, ensuring homeostasis, but preventing its use as an absorptive surface. (B) In the osmoconforming hagfish, the skin epithelium is permeable to salt and water, facilitating amino acid (AA) absorption.

and the internalisation of exchange surfaces such as those that perform gas transport and nutrient absorption (Fig. 1; Glover et al., 2013). This is an outcome with limited negative consequences for nutrient absorption, given the relative lack of opportunity for nutrient uptake across the skin in freshwater and terrestrial settings, and one that facilitates the exploitation of aquatic environments that vary in salinity and terrestrial settings where water conservation is a necessity.

The physiology of feast and famine

By virtue of a scavenging feeding mode, coupled with the infrequency of carrion falls, hagfishes may go long periods between meals (Foster and Moon, 1986). This intermittent feeding is akin to that observed in hibernating/overwintering animals and sit-and-wait terrestrial predators such as the pythons. In these species, periods of digestive quiescence result in a reduction in intestinal form and function, followed by a period of rapid upregulation when a meal is consumed (Secor, 2008). The remarkable functional and structural plasticity of intermittent feeders offers significant insight into the flexibility of physiological systems, and the costs and benefits of regulatory mechanisms.

Recent studies on the effects of re-feeding hagfish after a period of fasting have shown, however, that while changes in metabolic rate and gut structure and function occur, the magnitude of these changes is not of the scope observed in other species with extended fasting periods (see Fig. 2; Weinrauch et al., 2018; Glover et al., 2019). Instead, the changes observed upon consumption of a meal are very much in line with those seen in regular feeders. There are many possible explanations that could account, to some extent, for the comparatively small post-prandial response of hagfishes, ranging from the length of experimental fasting, the relative importance of scavenging (and therefore periodicity of meals) as a feeding mode (see below) and meal size. However, it seems likely that hagfishes simply do not exhibit the rapid and significant changes in metabolic rate, gut structure and physiological function

to the extent observed in the better-studied intermittent feeders. This lack of physiological plasticity is interesting; what physiological advantages, if any, are achieved by remaining relatively 'primed' to feed, in contrast to those species that undergo significant structural and functional flux? It is possible that the ability of a hagfish to absorb dissolved nutrients across the skin, coupled with its close association with benthic sediments rich in organic material, may facilitate a baseline level of passive nutrient absorption during periods of quiescence. This is supported by data that show epidermal amino acid uptake is not upregulated during immersive feeding, suggesting that this is a constitutive pathway (Glover et al., 2016). Thus hagfishes, at least those that burrow into or rest upon sediments, may be constantly acquiring dissolved nutrients from their environments, which could explain their relative lack of physiological change upon presentation of a solid meal.

The bad: amenability to experimental manipulations Inducing feeding

For any experimental biologist interested in digestive form and function, it is critical to be able to modulate the fed state of the test organism. Unfortunately, hagfishes are notoriously difficult to feed in captivity. This phenomenon was noted in some of the very earliest studies of hagfish (e.g. Worthington, 1905), and has been frequently observed in our own research. In one case, we attempted to feed the same group of Pacific hagfish (*Eptatretus stoutii*) regularly over an 11-month period. Despite a wide variety of food items presented (multiple fish species of various freshness, livestock offal, beef liver, chicken hearts, squid), feeding could not be induced (A.M.W., personal observation). More generally, we have noted that if food is withheld from hagfishes immediately following their capture, there is a narrow period after around 3 weeks in captivity during which feeding success seems to be greatest, with diced squid being the best feeding inducer (C.N.G. and A.M.W., personal observation). It is also notable that hagfish feeding is more likely to be successful if they are fed in a group (e.g. Strahan, 1963;

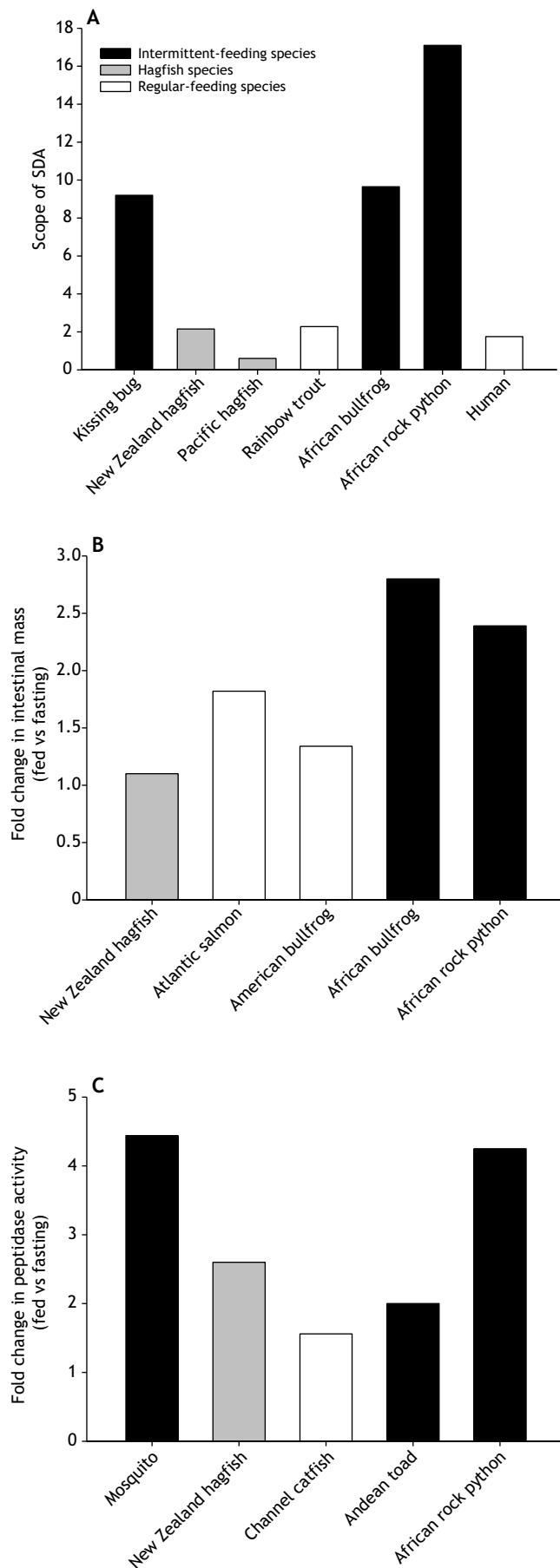


Fig. 2. Effects of feeding on metabolic, structural and functional aspects of digestion in hagfishes, intermittent-feeding and regular-feeding species. Effect of feeding on metabolic rate [scope of specific dynamic action (SDA) effect; see Glossary] (A), gut mass (B) and peptidase activity (C) in fed versus fasted regular-feeding animals (white bars), intermittent-feeding animals (black bars) and hagfishes (grey bars). Data collated from: Billingsley and Hecker, 1991; Bradley et al., 2003; Day et al., 2014; Glover et al., 2019; Krogdahl and Bakke-McKellep, 2005; Naya et al., 2009; Ott and Secor, 2007; Secor, 2005; Secor and Diamond, 1995; Tataranni et al., 1995; Thorarensen and Farrell, 2006; Weinrauch et al., 2018.

A.M.W., personal observation). This has been attributed to the greater chemosensory signal produced once a dietary item has been initially attacked. However, this is not always successful, as noted by the case study above, where even large amounts of a decaying food source were insufficient to induce feeding. This suggests other, as yet undetermined, factors may drive the recruitment of feeding in hagfishes (see below).

One work-around that researchers have employed is to assume that freshly captured hagfish are in the fed state (e.g. Glover et al., 2016; Wilkie et al., 2017). Experimental hagfish are caught using baited traps, which they enter, but cannot escape from. Consequently, all hagfishes in the traps have been actively seeking food and most have been feeding (as evidenced by the presence of food in the guts of most animals; C.N.G. and A.M.W., personal observations). The drawback of this approach is that the effects of capture stress (and the possibility of recent slime production; see below), may complicate interpretation of resulting data.

Even if hagfishes can be encouraged to feed naturally, there are often issues with being able to quantify their meal size, a measure that is important for comparing digestive metrics across the literature. Hagfishes have the unusual habit of gorging themselves to the extent that food emerges from the anus, relatively undigested, even while the animal is still feeding (Strahan, 1963; Baldwin et al., 1991). An alternative approach to remedy the fussy eating habits of hagfishes, and to ensure that gorging does not induce ‘anal leakage’, is to feed by gavage (see Glossary; Glover et al., 2019). However, the introduction of a liquefied diet can eliminate many of the key digestive processes that may be of interest (i.e. the metabolic costs of feeding, and the mechanical and chemical processes involved in the initial digestion of a food bolus). Furthermore, post-gavage handling can induce post-feeding problems, such as those described below.

Post-feeding manipulation

Assuming that a hagfish can be induced to feed, ensuring that the animal stays ‘fed’ can also be problematic. Hagfishes do not have tightened control of their anal musculature, and thus any form of post-feeding manipulation (e.g. anaesthetisation, handling) can result in the loss of gut contents (A.M.W., personal observation). Vomiting after gavage may also occur (C.N.G., personal observation). This means that any manipulations that require repeated handling (e.g. caudal blood puncture sampling over time) are difficult, if not impossible, to perform. An approach to minimise handling is the use of indwelling cannulae or probes; however, these techniques have limited utility in hagfishes.

Surgical implantation of cannulae or probes has proved invaluable to physiologists, facilitating the sampling of body fluids and/or measurement of changes in cardiac parameters. The data collected through such devices provide insight into the effects of environmental change and the regulatory mechanisms that form an organism’s response to that change. For example, the study of digestive processes via indwelling instrumentation in teleost fish has

facilitated measurement of postprandial blood flow changes (Axelsson et al., 2002), and evidence of an alkaline tide following feeding (see Glossary; Bucking and Wood, 2008). However, successful cannulation for repeated blood sampling is not easily achieved in hagfishes because of their very low blood pressure (Forster et al., 1991). Furthermore, the presence of the extensive blood sinus system (see Glossary) complicates analysis, in that the composition of sinus blood can be quite distinct from that of the main circulatory system (Glover et al., 2017). Although some authors have utilised instrumented hagfish (e.g. with implanted cannula in major vessels and chambers of the heart for studies of cardiac function; Davie et al., 1987; Cox et al., 2010), it is noteworthy that success is complicated by the knotting behaviour and other body contortions of hagfishes.

The slimy: the confounding role of hagfish slime

The complications associated with studying hagfishes are perhaps best encapsulated by examination of hagfish slime. The ability of this group to produce copious quantities of a thick gelatinous slime upon provocation is perhaps their most defining quality. In fact, a 60 g *E. stoutii* can theoretically produce around 24 litres of slime in a single event (Fudge et al., 2005). This slime has a number of remarkable properties with potential applications in a wide range of industries (Fudge et al., 2010), but for a hagfish itself it is principally considered an anti-predator mechanism. For example, a baited camera study recorded video footage of attempted hagfish predation events that did not end well for the aggressors (see Movie 1 in Zintzen et al., 2011).

For experimental biologists, slime can be a major complicating factor. Any disturbance of a hagfish can result in a sliming event. This includes the simplest of tasks from the experimental biology toolbox, such as removal of a hagfish from a holding tank or a change in water. If slime production occurs during the exposure of the hagfish to an experimental medium where composition or volume is critical, the reduction in volume and/or composition of the water, or at worse, the complete gelatinisation of the medium, has obvious negative consequences for a successful experiment.

Furthermore, the physiological state of the animal is also likely to be significantly altered following a sliming event. We are not aware of any studies that have specifically examined the metabolic and cellular resource costs of hagfish slime production, but given that stored slime accounts for around 3 to 4% of the total body mass (Fudge et al., 2005), it is likely to be substantial. As there is a limit to the extent and frequency of slime production (Schorno et al., 2018), some researchers have employed a methodology of handling the hagfish until slime production is exhausted before conducting experiments. This is not recommended because of the obvious confounding effect that this has on the biochemistry and physiology of the animal, and consequently, the quality of data produced. In our experience, sliming can be circumvented by very gentle handling, and especially by avoiding human contact with the animal, which hagfishes appear to be particularly sensitive to.

From the perspective of digestive physiology, the slime of hagfish appears to have a number of intriguing roles. Some researchers have noted the presence of slime on carcasses attended by hagfishes, an observation that could have important ecological consequences. For example, Tamburri and Barry (1999) noted that some invertebrate scavengers preferentially feed on the hagfish slime rather than a decaying food source. Consequently, this could reduce competition for the carrion, thus maximising the capacity of the hagfish to exploit it. The slime could also act as a deterrent to some scavengers, to a similar advantage. Even more intriguing is the

discovery that hagfish slime and epidermal mucus has been shown to exhibit amylase (Adam, 1963) and proteolytic (Subramanian et al., 2008) enzyme activity. This raises the possibility that at least one component of hagfish digestion occurs externally, in a similar manner to the bioactive compounds in the saliva of blood feeders, which begin digestion of cellular material prior to ingestion (e.g. lamphredin in sea lamprey; Lennon, 1954). This hypothesis requires further investigation and could be supported by studies that address the possibility of integumentary glucose transport, and research determining that enzymatic activity in the slime is not an artefact of the handling used to provoke the slime response. Hypothetically, however, the capacity to convert nutrient multimers down to more simple forms would complement the ability of hagfishes to absorb nutrients across their skin and gill surfaces.

Critical knowledge gaps

What are the endocrine mechanisms regulating hagfish feeding physiology and behaviour?

Suppression of appetite, which is a phenomenon that must occur in hagfish between feeding bouts, requires significant hormonal control. In the intermittently feeding python, cholecystokinin (CCK) is a known mediator of satiation (Secor et al., 2001). This hormone has been detected in hagfish tissues (e.g. Vigna, 1979), and porcine CCK has been shown to activate intestinal lipase secretion in hagfishes (Vigna and Gorbman, 1979). This action could signal a switch towards lipids as a fuel source and is consistent with a move from carbohydrate to lipid metabolism during fasting in this group (Emdin, 1982). This switch is supported by large and diffuse tissue lipid deposits that could accommodate lengthy periods without feeding (Weinrauch et al., 2019). Additionally, it has been observed that serotonin is produced by, and has biological effects in, hagfishes (Bernier and Perry, 1996). While specific actions of serotonin on hagfish digestive functions have not been investigated, in other animals this hormone is released in the presence of food and results in behaviours that facilitate meal attendance (Sawin et al., 2000). Serotonin also activates muscles and neurons involved in feeding, controlling an animal's perception of nutrient availability (Niacaris and Avery, 2003). These endocrine factors could be the missing links in understanding fasting periods in hagfishes and their reluctance to feed in captivity.

How do hagfishes sense feeding opportunities?

One of the most intriguing mysteries surrounding hagfish feeding is their ability to sense a feeding opportunity. Multiple studies have observed that hagfishes appear rapidly, and in large numbers, at baited camera traps (e.g. Collins et al., 1999; Martinez et al., 2011). However, in laboratory studies, they may respond slowly, or not at all, to food cues placed in close proximity (e.g. Worthington, 1905; Strahan, 1963; A.M.W., personal observation). While in some cases this may reflect a lack of 'hunger', it has led researchers to question the mode by which carrion and/or prey items are sensed.

There is some evidence that olfactory and chemosensory cues may play an important role in food detection by hagfish. For example, it has been observed that feeding behaviour is related to the concentration of amino acids provided in a feeding stimulus (Tamburri and Barry, 1999). Supporting this, the presence of amino acids in water perfused through the hagfish nostril does generate a neurological response (Døving and Holmberg, 1974). However, the threshold of this effect ($100 \mu\text{mol l}^{-1}$) is significantly higher than the concentrations likely to reach a hagfish any significant distance downstream of a food source. Whether olfaction plays a role in

real-world feeding initiation scenarios is a key knowledge gap, and requires further investigation.

An intriguing possibility for food sensing is the presence of the Schreiner organs. These chemosensory cells are unique to hagfishes, and although they appear similar in morphology to vertebrate taste buds, they are functionally distinct (Braun, 1996). Schreiner organs are widely distributed along the body surface, as well as within the nasal canal, with the highest densities present on the tentacles, trunk and tail (Schreiner, 1918; Braun, 1996). The neural requirement for these organs is significant, with 10% of medullary volume dedicated to Schreiner organ operation (Ronan, 1988). While these organs are obviously of great import in terms of hagfish sensory capabilities, there are no data on their potential stimuli, their sensitivity or their biological role. The possibility that these putative chemoreceptors function in food detection is intriguing, yet completely unstudied.

How important are different feeding modes?

Recent research has focussed on hagfishes as opportunistic scavengers, undergoing extended periods of quiescence in between meals. However, this is a simplified vision of hagfish feeding. Indeed, analyses of gut contents (see Glover and Bucking, 2016) and observational evidence from baited cameras (Zintzen et al., 2011) show that hagfishes may also be active predators of fish and invertebrates. However, the extent to which different feeding modes are employed is not known. For example, there are two studies that have used stable isotopes to provide a snapshot of nutrient sources in the hagfish *Eptatretus cirrhatus*. These studies produced distinct outcomes, with one concluding this species gained nutrition primarily through scavenging (McLeod and Wing, 2007), while the other suggests predation is the main feeding mode (Zintzen et al., 2013). The factors driving these intraspecific differences remain unknown, but mode-switching is likely to be shaped by habitat depth, frequency of carrion falls, presence of carrion competitors, variations in basal metabolic rates (e.g. seawater temperature, mass-specific oxygen consumption) and differences in the permeability of integument surfaces for direct nutrient absorption. These factors can all differ between populations and life-stages within a species, but could also drive differences in feeding modes between species. Knowledge of feeding mode is, however, critical both for designing experimental studies, and for contextualising and interpreting the results derived from such research.

How distinct is the physiology of different hagfish species?

Of the 82 hagfish species, only a small number have been subjected to investigation (Fig. 3). With respect to experimental research focussed on feeding and feeding-related processes, the species coverage reduces further (*E. stoutii*, *E. cirrhatus* and *Myxine glutinosa*). Even from these limited investigations, there is sufficient circumstantial evidence to suggest that species differences in aspects of feeding physiology are likely to be significant. There is, therefore, a need to greatly expand our knowledge of how key components of feeding behaviour, feeding ecology and digestive and absorptive processes, vary across this group. Until such studies are conducted, care should be taken when interpreting results of studies on one species as being representative of hagfishes in general.

Conclusion

Hagfishes display physiological characteristics that are unique and/or unusual with respect to their feeding behaviour, their acquisition and processing of nutrients, and their regulation of feeding-related physiology. Most intriguing are the testable hypotheses that studies

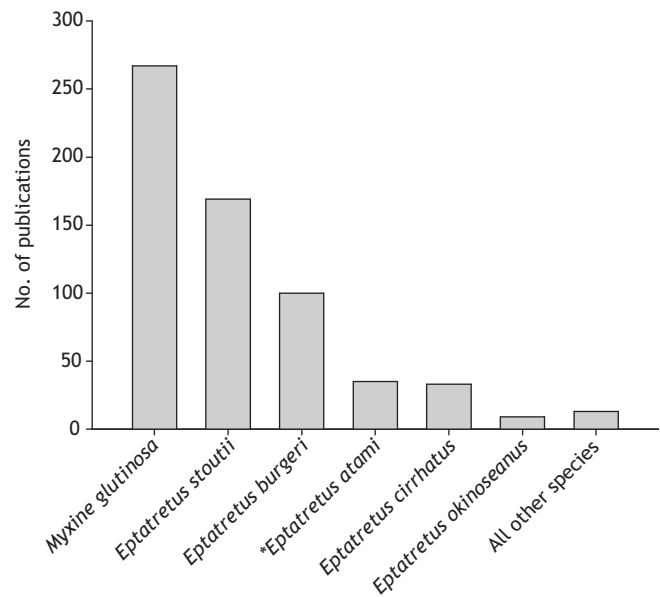


Fig. 3. Occurrence of different hagfish species as experimental subjects in published literature. Manually curated using Web of Science (up to February 2019), limiting records to primary publications examining structural or functional aspects of organismal biology. *Previously known as *Paramyxine atami*.

to date have generated, suggesting that the next few decades of experimental research on this group offer significant promise for major gains in our understanding of hagfish biology (see Box 2). However, these future studies must overcome the pitfalls of hagfishes as an experimental organism, or at the very least must carefully detail the effects of phenomena such as sliming and feeding induction approaches on the observed outcomes. Advances in our understanding of hagfish feeding ecology and the physicochemical properties of hagfish habitats will greatly facilitate such studies and will ensure that experimental work is founded on real-world feeding scenarios.

Box 2. The peritrophic membrane and gut microbiota

One trait hagfish share with invertebrates, but which is lacking in all but a few vertebrates, is the presence of a peritrophic membrane (PM) (Adam, 1966; Nakashima et al., 2018). This is essentially a chitin-based mesh 'bag' which encapsulates the food bolus and, at least in invertebrates, has roles in digestion (e.g. facilitates entry and concentration of digestive enzymes) and protection (protects the gut epithelium against toxins and/or physical damage originating from the food bolus) (Engel and Moran, 2013). Another key role of the PM in invertebrates, which lack an adaptive immune response, is that it segregates microbes from the gut epithelium, thus preventing the interaction of microbes with the animal itself. This differs from the situation in mammals. Because of an adaptive mucosal immune system, the mammalian gut surface is capable of tolerating microbial exposure, and indeed may harness these microbes for a variety of functions that contribute towards gut and overall animal health (e.g. Maynard et al., 2012). A consequence of these different strategies is that the gut microbiomes of species with a PM are generally more reflective of the environmental microbial communities, while gut microbiomes of species without a PM can be relatively independent of the ambient microbiome (Nakashima et al., 2018). Hagfish do possess a form of adaptive immunity (Li et al., 2016), and so are theoretically capable of cultivating a specific microbiome with functional benefits to the animal. However, to date, there are no studies on the hagfish gut microbiome, and nor have the possible nutritive and digestive functions of the hagfish PM been investigated.

Competing interests

The authors declare no competing or financial interests.

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