Shaping Brain Sexuality

Male plainfin midshipman fish exercise alternative reproductive tactics. The developmental trade-offs involved shape two brain phenotypes

Andrew H. Bass

Viewed from the perspective of an evolutionary biologist, life is a game whose object is to maximize the number of individuals carrying your genes into subsequent generations. Reproductive strategies vary with the species, but they always represent some kind of trade-off. Some animals try to beat the odds by producing as many offspring as possible, leaving little time or metabolic energy to care for them. Other animals try to maximize the survival rates of those they do produce by having fewer young, but tending to them until they go off on their own. Particular strategies adopted by animals to win this game have evolved as a response to the specific selection pressures on particular species.

Biologists have come to appreciate that selection pressures may act on individuals within a species, so that different individuals of the same sex may employ very different reproductive tactics. This view comes in part from studies of teleost fishes, where males may engage in one of several alternative reproductive tactics. The sequentially hermaphroditic reef fishes, such as wrasses, sea bass, gobies, parrotfish and anemonefish include individuals that can permanently change their sex. Behavioral sex change begins within minutes of a social cue, and complete sex change can be achieved within days. Other teleosts, such as sunfish, swordtails, platyfish, salmon and the plainfin midshipman—the fish I study in my laboratory—cannot change sex. Rather, in these species, individual males develop into one of two types, or morphs.

For the midshipman, type I males are the larger of the two, and the only morph capable of attracting females.

Type I males build the nests in which females deposit their eggs and attract the females with their almost indefatigable humming, which has earned them the nicknames "California singing fish" and "canary bird fish." To acquire these abilities, type I males take longer to reach sexual maturity than do the second male morph, the type II males.

Type II males may become sexually mature earlier than type I's, but they

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Figure 1. Underneath rocks along the intertidal and subtidal zones of the western coast of North America from Canada down to northern California, type I male plainfin midshipman fish build their nests. These fish are teleosts, many species of which manifest unusual reproductive strategies. Some are hermaphrodites, able to alter their sex in a matter of minutes. Others, such as the midshipman, have two forms of males—the nest-building type I, and the "sneaker males," or type II. Type I males also attract the females to the nest, coax them to lay their eggs and guard the nests, as they can be seen doing above and to the right. Type II males, on the other hand, do not build nests or attract females on their own; they merely sneak into the type I's nests and deposit their sperm. The differing behavior of these two distinct reproductive morphs provides neuroscientists with a rare opportunity to study whether and how behavioral differences translate into differences in the brains of these fish. (All photographs courtesy of Margaret Ann Marchaterre.)

lose something in the bargain. They are smaller and have never been found to build nests or attract females. Their reproductive strategy is to sneak into the Type I's nest or lie perched outside the nest's entrance and deposit their sperm there, earning them the nickname of "sneaker males" or "satellite males."

Having two distinct male forms—each exhibiting distinct behaviors—presents neurobiologists such as myself with a unique opportunity to study a brain-behavior relationship. At the root of this issue is whether behavioral differences translate into differences in the structure and function of the nervous system. I have found that in fact they do.

The behavioral trade-offs exhibited by the two male morphs essentially reflect the sexual phenotype of the nervous system, which in turn directs the expression of an adult individual's sexual behavior. Armed with this knowledge, my colleagues and I have been exploring the factors that shape the sexual differentiation of the type I and type II brains throughout the animal's sexual development. We have focused our efforts on understanding how the structure and function of the brain might be shaped by early events in development that involve trade-offs between individual characters, such as growth rate and age or size at sexual maturity. An understanding of how and why early developmental events lead to alternative phenotypes for individuals within a species provides fertile ground for examining the linkages between neurobiology and behavioral ecology within a modern evolutionary framework.

Nests and Songfests

Along the western coast of North America, from southern Canada into northern California, from late spring
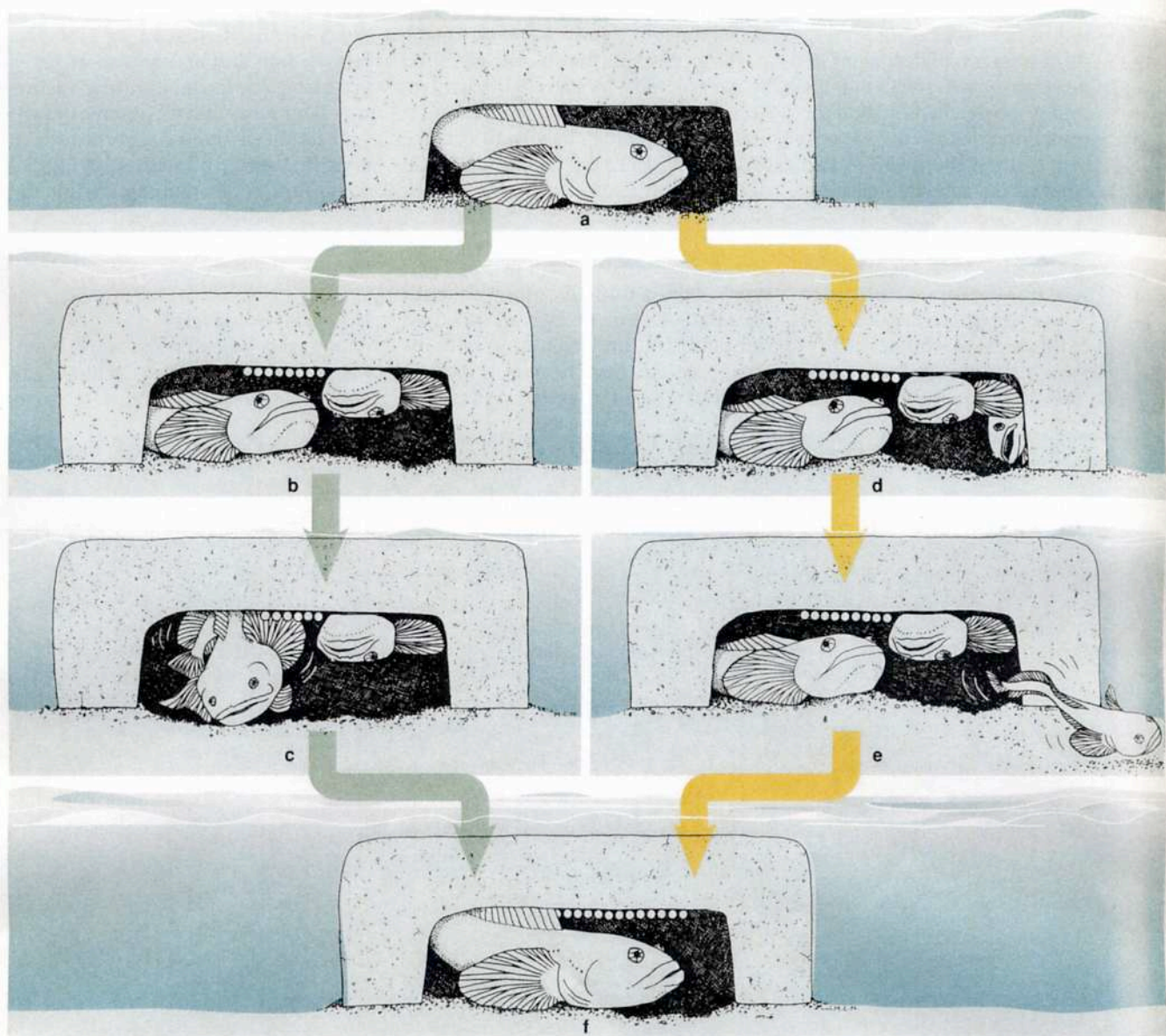
Figure 2. Spawning behavior of type I male plainfin midshipman differs dramatically from that of type II. A type I male generates advertisement calls in the form of low-frequency hums from inside his nest after nightfall (a). The male stops his humming after a gravid female enters the nest and they spawn. The female deposits eggs on the roof of the nest (b). The type I male rolls and quivers and releases sperm near the eggs (c). A variation on this sequence takes place when a type II male enters the picture. If he can get inside the nest, the type II male (far right) sneak spawns (d). Otherwise, the type II male releases sperm while fanning water towards the nests's opening (e). After egg laying is completed, the female leaves the nest, and the type I male remains to guard the eggs (f). He hums again the next evening to attract another female, and the sequence begins all over again. This sequence is based on a series of photographs of captive, reproductively active specimens that have taken up residence in artificial nests in aquaria. (Illustration adapted from Brantley and Bass 1994; courtesy of Margaret Nelson.)

through summer, the "song" of the male plainfin midshipman can be heard after nightfall. This song, really a low-frequency hum, may drone on to human ears, but it is highly attractive to female midshipman fish, who seek out the singers and mate with them. The males await the arrival of the females in nests that they build under rocks in the intertidal and subtidal zones. Females deposit their eggs and leave the male to guard the nest soon after spawning.

Not all male midshipman are vocal lotharios or nest builders. As mentioned before, these activities are the sole province of type I males. Richard Brantley, a former graduate student in my lab who is now at Vanderbilt University discovered that type II males exploit the type I's reproductive tactics. As mentioned earlier, they lie perched outside of or sneak into a type I male's nest and shed sperm in a competition with the type I male for the eggs. It is unknown whether type II males remain affiliated with one or several type I nests.

In addition to behavioral differences, each reproductive morph also has a characteristic suite of morphological traits. On average, type I males are about two times longer and eight times heavier than are type II males at the time of sexual maturity. Surprisingly, although they are smaller overall, type II males have the advantage in gonad size. The average ratio of gonad to body weight in type II males is nine times greater than in type I males. Type II males may therefore invest up to 15 per-

cent of their weight in testes, compared with only one percent in type I males.

Gravid females resemble type II males in having a large gonad-to-body weight ratio. Both gravid females and type II males have a distended and firm belly, reflecting the large size of their gonads. In fact, it is easy for the untrained observer to mistake a type II male for a small, gravid female. Adding to the confusion, type II males and females are similarly colored. Although the backs of all three reproductive morphs are olive-gray, the bellies of the fish differ during breeding season. Type I males are typically light to dark gray on the underside, whereas type II males are mottled yellow. Gravid females have a bronze or golden color on their bellies, and spent females are more like type II males in their appearance.

The females, for their part, apparently select only one type I male to mate with each season. The number of eggs per female increases with body size and may approach 200. Each female leaves her entire clutch in the nest of the chosen male. Midshipman have large eggs, approximately 5 millimeters in diameter, which are attached by an adhesive disk to the roof of the nest. Embryos develop upside-down while attached to the yolk sac. After the fry hatch, they remain in the nest. But during their early pre-reproductive months, juveniles aged 5–12 months are found only in eelgrass beds, where adult morphs are also occasionally found. Whereas females apparently mate with only one male per season, type I males, and presumably type II's, mate with several different females. Each nest typically contains several thousand eggs—obviously originating with many different females.

Nesting type I males generate two major classes of vocalizations. They make short grunts, which, along with their large body mass, form an effective threat to any potential intruder males. But the sounds that have been of particular interest to members of my laboratory are the mating sounds, the monotonic hums that can last from minutes to over an hour at a time. Those of us who study this phenomenon know that these are mating calls by observing the fish in experimental situations. For example, Jessica McKibben, a graduate student currently in my lab, played computer-synthesized acoustic signals that approximate these hums to females, who are then attracted to underwater speakers in outdoor aquariums. Simulated grunts, on the other hand, seem to do nothing for the females. The hum may help females select the best male to mate with, or it may just serve as a beacon for females looking for nest sites. It may also attract the type II males to these sites. Type II males, like females, do not produce hums at all, and only produce the occasional grunt in non-spawning situations.

Just as the differences between the two male types in their acoustic repertoire are quite pronounced, so too are the differences in the level of development of the organs that produce those sounds. The vocal organ of a midshipman consists of a pair of sonic muscles attached to the walls of the swimblad-

Figure 3. Seen from above, the three reproductive morphs—type I males (large fish, *lower right*), type II males (four smaller fish, *left and center*) and females *(topmost fish)*—appear the same olive-gray. The undersides of the fish differ during breeding season. Type I males are typically light to dark gray on the underside, whereas type II males are mottled yellow. Gravid females have a bronze or golden color on their bellies, and spent females are more like type II males in their appearance.

sexually polymorphic traits	type I male	type II male	female
nest building	yes	no	no
egg-guarding	yes	no	no
body size	large	small	intermediate
gonad-size/body-size ratio	small	large	large(gravid), small(spent)
ventral coloration	olive-gray	mottled yellow	bronze (gravid), mottled (spent)
circulating steroids	testosterone, 11-ketotestosterone	testosterone	testosterone, estradiol
vocal behavior	hums, grunt trains	isolated grunts	isolated grunts
vocal muscle	large	small	small
vocal neurons	large	small	small
vocal discharge frequency	high	low	low

Figure 4. Traits of type I and type II males differ markedly. In many respects, type II males more closely resemble females than they do type I males.

der. Contracting the muscles causes the swimbladder to act like a drum—which produces the type I male's low-frequency hums and grunts. One would expect the huge disparity in the vocal capabilities of the two male morphs to be reflected in differences in the sonic musculature. Indeed this is the case.

The ratio of vocal muscle to body weight is six times greater in type I males than in type II males or in females. Furthermore, type I males have four times as many muscle fibers and these are five times larger in diameter than those of type II males and females. Margaret Marchaterre, an electron microscopist in my lab, and I discovered that the disparities are evident even at the subcellular level.

For example, the Z-lines of muscles, the points at which the actin filaments of muscle overlap, which show up under the electron microscope as a dark band, are much wider in the sonic muscles of type I than in type II males or females. The reservoir called the sarcoplasmic reticulum, which contains calcium ions required to mediate muscle-cell activities, is more highly branched in type I males. In addition, the muscles of these males contain vastly higher numbers of the subcellular energy-producing mitochondria than do the muscles of type II males and females. All of this—both the gross appearance of the muscle and the subcellular features—suggests that the sonic muscles of type I males are in every way better equipped than are the muscles of type II males and females

to sustain the continuous singing. (Imagine trying to sing without stopping for over an hour.)

The relative differences in muscle development in the two male morphs are impressive, but there is nothing especially surprising about it given the vocal prowess of the type I males. My interest as a neuroscientist is in determining the neuronal input into this differential behavior. To do that, my colleagues and I looked at the brains of these animals to see whether the difference in muscle size and use in the two male morphs is in any discernible way reflected in their control by nerve cells in the brain.

Brainwork

Before my colleagues and I could start to parse differences in the brains of type I and type II males, we had first to identify the neuronal pathway controlling vocalizations. In this task we were aided by the recent discovery in my lab that a tracer compound called biocytin, which consists of the amino acid lysine and biotin, a naturally occurring protein in neurons, turns out to be perfect for delineating entire circuits in the midshipman's brain. We learned that we could apply biocytin crystals to the cut ends of the motoneurons, the cells that innervate and stimulate the sonic muscles, and the biocytin would be carried backwards from the nerve ending along an axon to its parent cell body. Biocytin also completely filled the arbor of dendrites that extends from the cell body and receives inputs from other neurons. Furthermore, the biocytin did not stop in the first cell it encoun-

tered. Rather, it crossed into the synaptic space between that cell and the end-terminal of the one before it in the circuit. The biocytin travels backwards this way, all the way up to the first cells in the brain that we have so far identified as the sites that initiate vocalizations. Since the biocytin stains the cells brown, my colleagues and I could actually see the entire vocal neuronal circuit required for stimulating the sonic muscle. Biocytin staining confirmed what we had previously found using standard neurophysiological techniques and helped us find new components.

The cell bodies of the sonic motoneurons that stimulate the sonic muscle lie in two sausage-shaped clusters on both sides of the midline of the midshipman's brain, close to the junction of the brain and spinal cord. In adults, about 2,000 cells are found in each cluster, or nucleus. Axons exiting from the sonic motor nucleus bundle together and leave the brain to form the sonic nerve, which stimulates the activity of the sonic muscle.

Robert Baker, at the New York University Medical Center, and I demonstrated that the sonic motoneurons in midshipman receive direct input from a set of pacemaker cells that lie just adjacent to the motoneurons. Each pacemaker neuron connects to motoneurons on both sides of the brain and fire in a constant rhythm, setting the pace at which the sonic motor cells fire. The rhythm set by pacemaker cells corresponds exactly with the rhythm at which sonic motoneurons stimulate the

sonic muscle. This in turn determines the frequency at which the muscles contract, which ultimately determines the pitch of the sound.

Our mapping studies with biocytin allowed us to discover another set of neurons in this circuit that had previously been unknown. We found a cluster of cells just in front of the sonic motor nucleus, which we called the ventral medullary neurons. These neurons form the major route connecting the two sides of the pacemaker–motoneuron circuit and so likely make a major contribution to coordinating the activities of both sides of the brain. This eventually leads to the simultaneous contraction of both sonic muscles.

We are just beginning to investigate the sensory stimuli that might activate the vocal motor system. One obvious candidate would be activation of this system by a neighboring midshipman's vocal signal. The inner ear of all vertebrates has a number of divisions. In the midshipman, the largest one is known as the sacculus, which is considered to be the main organ of hearing. It includes

a palette of sensory cells linked by the eighth nerve to neurons in the hindbrain, which are the first in a chain of neurons forming a central auditory pathway that extends through all levels of the brain. Deana Bodnar, a research associate in my lab, has recently identified neurons in a midbrain auditory nucleus that encode information that could be used to recognize differences between the hums of neighboring males. This discovery, together with other anatomical data collected from our biocytin studies, suggest that midbrain auditory neurons along with neurons of the paraventricular and tegmental nuclei may form a vocal-acoustic network, which provides a circuit for vocalizations to be elicited by the sounds of neighboring midshipman.

Having worked out the entire circuit, members of my lab group were in a good position to make comparisons of the brains of the different morphs to determine whether there were any obvious differences between them. The first thing we discovered was that male morphs and females possess identical

circuitry. We also found that they have the same ratio of nerve cells to body weight, so any differences in behavior could not be due to the number of cells.

We did find, however, that the pacemaker-motoneuron circuit in type I males fires at a frequency that is about 15 to 20 percent higher compared with type II males and females. This parallels sex differences in the frequency of natural vocalizations. We also found that the cell bodies, dendrites and axons are one to three times larger in type I males than in females and type II males. The junction between the nerve and muscle is also larger in type I males. Therefore differences within and between sexes in the organization of the vocal motor system depend upon a divergence in the morphological and physiological properties of individual nerve cells. It seems likely that the larger cells of the type I male are specifically adapted to fire more frequently and without attenuation for a longer period to support the activity of their much enlarged sonic muscle during prespawning periods of singing.

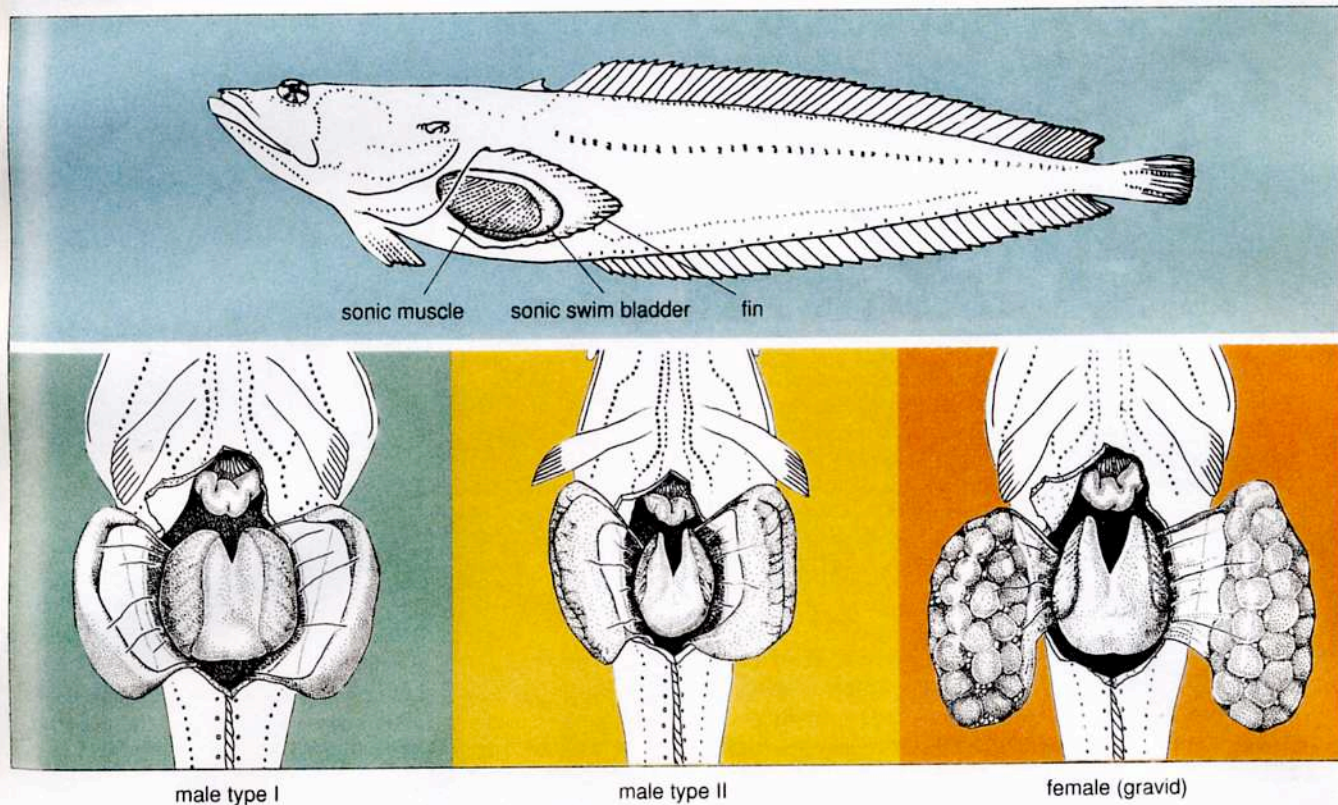male type I   male type II   female (gravid)

Figure 5. To attract females to their nests, type I males perform low-frequency hums throughout the night during breeding season. The vocal organ of the midshipman fish is a pair of sonic muscles attached to the walls of the swimbladder (top). Contracting the sonic muscles makes the swimbladder act as a kind of drum. Sonic muscles of type I males (bottom left) are extremely well developed in comparison with muscles from type II males (bottom center) or females (bottom right). The ratio of sonic muscle to body weight is six times greater in type I males than in the other reproductive morphs. In contrast, the gonad to body weight ratio is nine times larger in type II males and 20 times larger in gravid females than in type I males and juveniles. (Illustration courtesy of Margaret Nelson.)
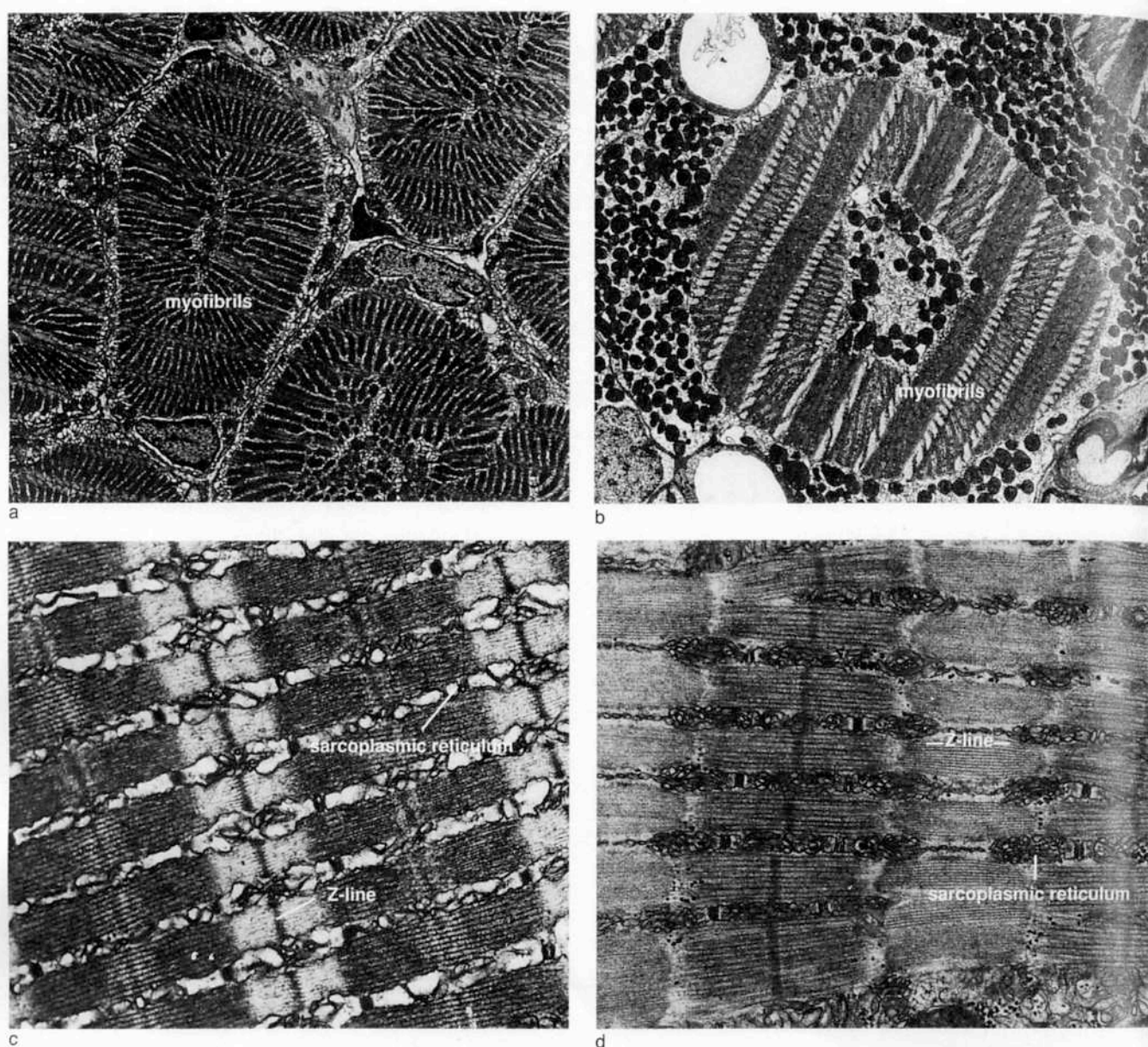
Figure 6. Morph-related differences in sonic muscles can be seen clearly in the electron microscope. In cross section, sonic muscles from type II males (*a*) and type I males (*b*) appear tubular. The myofibrils of juveniles, females and adult type II males are densely packed, surrounded by a thin rind of sarcoplasm, which contains the muscle-cell nuclei and a few mitochondria, which mediate muscle activity. Type I males have fibers with an inner doughnut-shaped core of myofibrils bordered by a large volume of sarcoplasm densely filled with mitochondria. When viewed in longitudinal section, additional differences between muscle fibers of type II (*c*) and type I males (*d*) become apparent. The width of Z-lines, the point at which the actin filaments of muscle overlap, and the degree of branching of sarcoplasmic reticulum are greater in magnitude in type I males. Females and juveniles resemble type II males. The bar scale is 6 micrometers in panels a and b, 0.8 micrometers in panels c and d. (Electron micrographs courtesy of the author; adapted from Bass and Marchaterre 1989.)

Getting Big

The studies performed by my colleagues and I thus suggest that alternative mating tactics among sexually mature males are paralleled by alternative phenotypes for the neurons in the relevant circuit. My colleagues and I wanted to learn the origins of these differences. We could envision at least two distinct scenarios.

In the first, juvenile males may transform into either type I or type II males. That is, both types of males follow mutually exclusive, nonoverlapping growth patterns. The other possibility is that the smaller type II males eventually change into type I males. To determine which of these was happening, my coworkers and I followed the development of these fish in the early juvenile stages just prior to and on into sexual maturity.

We exploited the properties of biocytin to map and trace the development of the neuronal vocal circuit in juvenile midshipman. These studies revealed that for type I males sexual maturation is preceded by growth of the mate-calling circuit and the sonic muscle. The size of motoneurons and the volume of the entire sonic motor nucleus, which likely reflects the growth of motoneuron dendrites as well, increase twofold just prior to the type I's sexual maturation. At the same time, the number of sonic muscle fibers increases by four.

With the onset of sexual maturity, the type I male experiences an additional, albeit more modest, growth in the size of

motoneurons. This, however, is coupled with a large increase in the size of pacemaker neurons although the size of this increase does not compare to that of motoneurons. At this time, the sonic muscle undergoes its major expansion—a remarkable fivefold increase in the size of the muscle fibers, which accounts mostly for the large increase in sonic-muscle weight in type I males. It was surpising to see that the largest increase in muscle growth followed, rather than coincided with, the largest growth in the neural circuit. The neurons in the ventral medulla show similar growth increments during both stages.

In contrast, the transformation from juvenile to type II male or to adult female is not accompanied by the dramatic changes seen for type I males in the sizes of vocal neurons and muscle cells. In fact, these cells change little or not at all as type II males and females mature. The sum of these findings suggests that type I males and type II males and females grow along alternative growth trajectories, at least as it concerns the neurons and muscles that determine morph-specific vocal behaviors.

As a next step in our research, my colleagues and I were interested in learning the rate at which each reproductive morph achieved sexual maturity. To do this, we had to be able to determine the precise age of the individual fish we studied, which turned out to be surprisingly easy.

The sacculus division of the inner ear of teleost fishes contains a structure called an otolith, which is mainly composed of calcium carbonate. As a fish grows, new layers of calcium carbonate are added to the otolith. These layers of calcium carbonate can be read, like the rings of a tree, to determine the individual's age. By reading the growth increments in midshipman otoliths, Ed Brothers of EFS Consultants in Ithaca, New York, and I have shown that type I and type II males not only overlap in age, but type II's become sexually mature earlier than do type I males. This finding, we believe, adds support for our hypothesis that alternative male morphs in midshipman fish adopt nonsequential, mutually exclusive growth patterns during their first year of life.

An Early Start
At this point in our research, we were reasonably convinced that the path leading to one or another male morph was set very early in the animal's development. We know that for fish, as for all vertebrates, hormones are necessary for the development of secondary sexual characteristics. We were interested to see whether differences between type I and type II males relating to their reproductive strategies might be reflected in hormonal differences acting during maturation.

A number of studies have indicated that the hormonal cascade leading to sexual maturation is initiated in a part of the forebrain known as the preoptic area. Neurons in this region release a neurochemical called gonadotropin-releasing hormone (GnRH). GnRH is a 10 amino-acid-long peptide that has been identified in a wide range of vertebrate
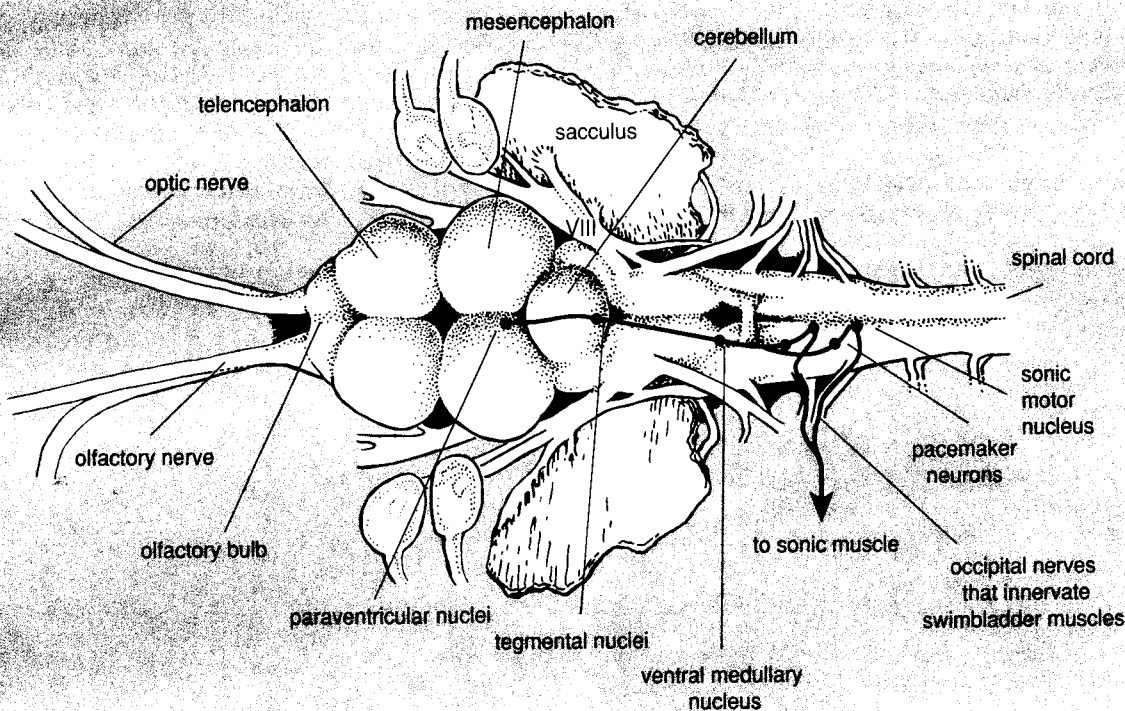

Figure 7. Differences in the vocal-motor circuit in the brain controlling vocalization parallel behavioral differences between the morphs. Here a schematic illustrates the entire circuit beginning in the brain. The initial stimulation comes from sites in the mesencephalon and hindbrain called paraventricular and tegmental nuclei. These stimulate ventral medullary neurons, which in turn stimulate pacemaker neurons. Pacemaker neurons fire at a set frequency equal to the one at which the sonic motoneurons fire and the sonic muscle contracts. In effect, the pacemaker neurons determine the pitch of sounds made by midshipman fish. The axons of the sonic motoneurons bundle together and eventually form the sonic nerve, after exiting the brain via paired occipital nerve roots. The sonic nerve stimulates the sonic muscle on that side of the body. The activity of the two sonic nerves emerging from each side of the brain are coordinated by ventral medullary and pacemaker neurons. In this way, both sonic muscles contract synchronously. The circuit in the three reproductive morphs is the same as is the ratio of neurons relative to body weight. The neurons in the circuit of type I males have larger cell bodies, dendrites and axons than do those in the other two morphs. These features likely help the neurons of type I males fire frequently without fatigue or attenuation. (Adapted from an illustration by Margaret Nelson.)

species. Working with Dean and Tami Myers, Cornell colleagues now at the University of Oklahoma, our lab showed that the gene sequence encoding the GnRH decapeptide in midshipman is remarkably similar to the GnRH coding sequence in other fishes, amphibians, birds and mammals, including people. This finding argues for a highly conserved function for the GnRH peptide among all vertebrates.

GnRH stimulates another set of cells in the pituitary gland, a structure found at the base of the brain. Once stimulated, the pituitary releases a family of hormones called gonadotropins. Gonadotropins act directly on the developing gonads, be they female or male. The gonads, in turn, release steroid hormones—androgens, such as testosterones and estrogens—that stimulate the development of secondary sexual characteristics. In midshipman, one of the effects of the steroid hormones is to mediate the development of the vocal motor system.

One of our first projects was to compare the steroid hormones produced by the three reproductive morphs. Working with Brantley and John Wingfield from the University of Washington, we found that the different morphs did indeed produce different levels of the various hormones.

Testosterone is detectable in all three morphs, although at progressively lower levels, with type II males producing the highest levels followed by females. Type I males produce the lowest amounts. Estrogen (in the form of 17β-estradiol) is detectable only in females, but at much lower concentrations than testosterone.

In addition to these common steroids, teleosts produce a unique form of testosterone known as 11-ketotestosterone. On average, type I males have five times as much 11-ketotestosterone as they do testosterone; 11-ketotestosterone is undetectable in type II males and females. This hormonal distribution is similar in all teleosts with two distinct male morphs. It seems likely, then, that 11-ketotestosterone is more potent than testosterone in supporting courtship behaviors, such as humming.

We were also interested in knowing whether hormonal differences could explain why type II males reach sexual maturity earlier than type I males. Martin Schreibman and his colleagues at Brooklyn College first showed in platyfish, which also have two male morphs that differ in age at sexual maturity, that the fish start to become sexually mature when the GnRH cascade is initiated. Our studies of midshipman sexual development have yielded similar results. Matthew Grober, a former postdoctoral associate in my lab and now at Arizona State University, led a study that found that the number and size of neurons releasing GnRH increase as the animal is making the transition from juvenile to adult. At the point of sexual maturity, this region is equally as developed in all three morphs. That is, it is not the case that the more sexually precocious morphs have better-developed hormonal circuits. The difference, as indicated by otolith-aging studies, is that the cascade is turned on at least three to four months earlier in type II males and females than in type I males.

The question that remains for us to answer is whether these differences are genetic or environmental. When I began these studies, I believed firmly that the difference between type I and type II males was programmed into their genes. But recent studies with Christy Foran, a current graduate student in my laboratory, are casting some doubt on that assertion. We have some preliminary experimental data to suggest that the number of type II males produced is a function of population density. Our work suggests that under sparsely populated conditions, more type I males are produced. As the population density increases, so too does the percentage of type II males.

Alternative Male Morphs: Trade-Offs
Our work suggests that type II males reach sexual maturity earlier than type I males, but they remain physically and behaviorally immature with regard to their ability to vocalize. On the other
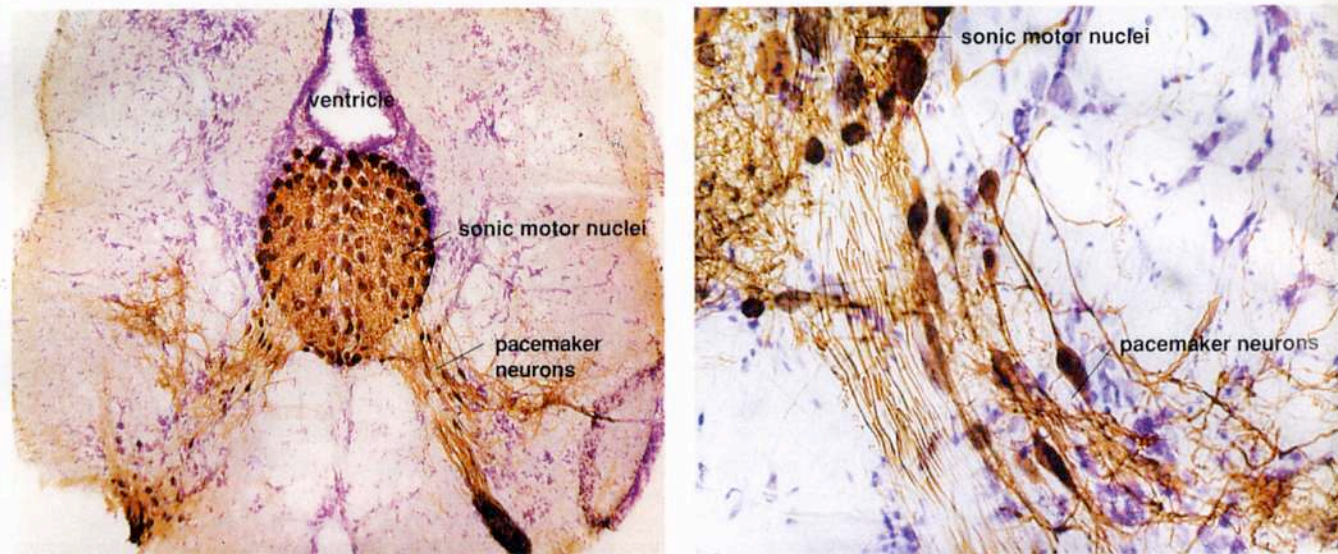

Figure 8. Neuronal circuitry of the vocal motor system can be traced using biocytin, a compound made of the amino acid lysine and the protein biotin. Biocytin crystals are applied to the cut ends of the axons innervating the sonic muscle. The compound is carried backwards to the cell bodies of these neurons, and further backwards still into the nerve cell innervating that one and on back to the first nerve cells in the circuit. In a low-power photomicrograph of the circuit in a type I male *(left)*, sonic motor nuclei, which contain the cell bodies and dendrites of the sonic motoneurons, appear brown when stained with biocytin. Also shown are the pacemaker neurons, which lie along the sonic motor nuclei. Biocytin transport results in extensive filling of the cell bodies, dendrites and axons of vocal pacemaker neurons, as shown in the right panel. (Photomicrographs courtesy of the author.)
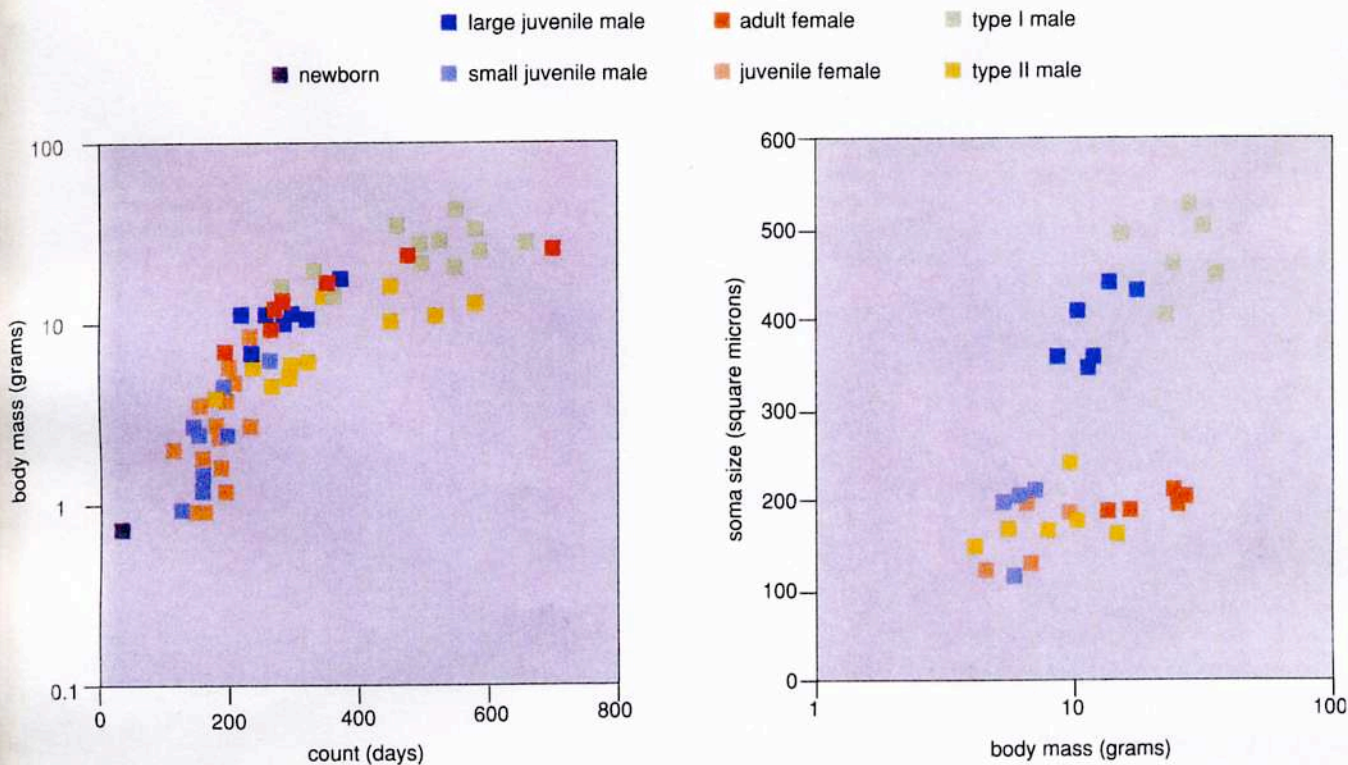
Figure 9. Alternative male morphs represent nonsequential, mutually exclusive life-history tactics, according to studies of their developmental trajectories. The age of fish can be determined by counting the growth increments on otoliths, calcium carbonate structures in the ear. The left graph includes data from 72 individuals representative of all juvenile and adult morphs. It is clear that type II males and females can reach sexual maturity earlier than do type I males and that one type does not change into the other. The right graph is a scatter plot of average values for the size of cell bodies of sonic motoneurons of juvenile and adult morphs. The data indicate alternate growth trajectories for the neurons that determine morph-specific vocal behaviors. This further supports the notion that type I and II males adopt mutually exclusive patterns of growth during their first year of life. (Adapted from Bass *et al.*, in press.)

hand, type I males delay maturation, but have a fully developed sexual behavioral repertoire.

In his book *Ontogeny and Phylogeny*, Stephen Jay Gould reviewed the extensive literature suggesting that the dissociation in time between sexual and physical maturity, referred to as heterochrony, is often characteristic of speciation events. But from our work, we see that heterochrony may also lead to behavioral innovation within a species. In a 1986 review article in the *Proceedings of the National Academy of Sciences*, Mary Jane West-Eberhard proposed a switch mechanism giving rise to alternative phenotypes within a species.

The evolutionary significance of a switch is that it determines which of an array of potential phenotypes will be expressed and, therefore, exposed to selection in a particular timespan and context. Insofar as one set of characters is independently expressed relative to another, it is independently molded by selection. Therefore, different covariant character sets evolve semi-

independently, taking on different forms in accord with their different functions. Like juvenile and adult forms, different alternative phenotypes of the same species may show dramatic differences in morphology, behavior and ecological niche. This is possible because once a switch mechanism is established, contrasting phenotypes can evolve simultaneously within the same genome—without reproductive isolation between forms.

It seems likely that the type II male morph developed under conditions of intense sexual selection, namely competition between males for access to females and nest sites. The switch mechanism is associated with a trade-off among midshipman males in the age and size at sexual maturity, as well as a multidimensional suite of secondary sexual characteristics. Thus whereas type I and II males share gonadal sex, they are highly divergent in behavioral, cellular, hormonal and vocal-motor traits. The convergence or monomorphism in behavioral and

physical traits between type II males and females reflects a common developmental pattern of trade-offs.

This implies that the type I male morph represents the ancestral behavioral state for male plainfin midshipman. This is supported by the available comparative data that show that other species of midshipman and their closest phylogenetic relative, the toadfish, have a single male reproductive phenotype resembling type I males. Therefore the most parsimonious conclusion is that the type II male morph represents a derived character state for this group of teleosts.

The close temporal onset of sexual maturity in type II male and female midshipman resembles many other teleosts. Graham Bell at McGill University shows in a review of approximately 100 freshwater species that the age at sexual maturity in males is close to or less than that of females. The exceptions are in species where males, like the type I midshipman morph, guard a territory or suitable substrate for a female's eggs. By adopting early maturity and thus an
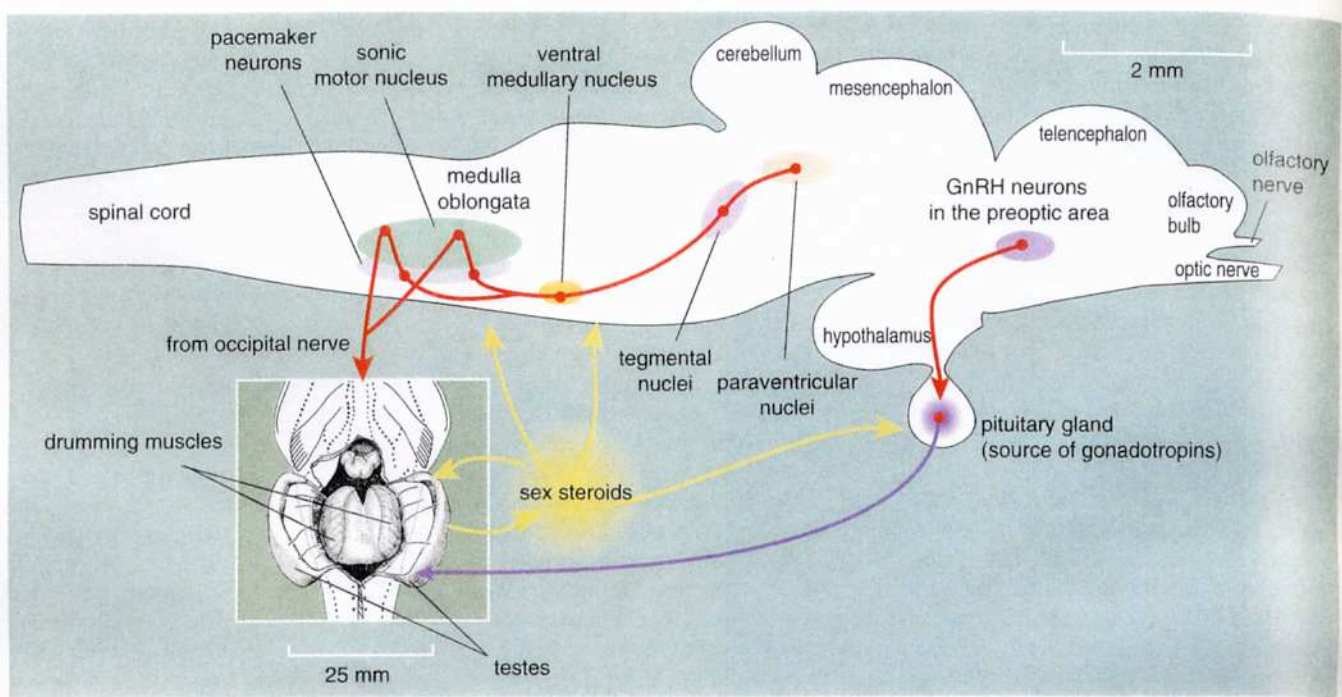
Figure 10. Proposed cascade of hormonal events leading to the expression of type I male, type II male and female reproductive morphs is shown in this schematic. Gonadotropin-releasing hormone (GnRH) is released from a region in the forebrain. GnRH stimulates the pituitary gland, at the underside of the midshipman brain, to release gonadotropins. Gonadotropins stimulate the gonads to release steroid hormones, such as testosterone. These steroids stimulate the differential development of secondary sexual characteristics, such as the ability to vocalize and hence the neuronal circuitry of the vocal motor system. Type I males have a type of testosterone, called 11-ketotestosterone, not found in the other two morphs. This hormone might be especially potent in the development of the sonic musculature and the neural circuitry. The hormonal cascade leading to sexual maturity is initiated three to four months earlier in sexually precocious type II males and females than it is in type I males. The contributions of ecological, behavioral and genetic factors to the activation of a GnRH-gate remain to be defined.

increased chance of surviving to become reproductively active, type II male midshipman essentially forgo direct competition with type I males to establish nest sites. Their investment in gonad development and in an expanded hormonal system is likely to be among the principal costs associated with early maturation.

Type I male midshipman derive at least two major growth-related benefits from delayed maturity. The first is increased body size, which would provide an advantage in combat and nest competition. The second is hypertrophy of a vocal motor system that generates vocalizations important, at the very least, for female attraction to nest sites and probably useful for intimidating other males. The principal costs for type I males in delaying maturity are the considerable structural and metabolic investment in physical and behavioral development and an increased chance of *not* surviving to sexual maturity.

Michael Taborsky at the Konrad Lorenz Institute in Vienna, Austria, points to external fertilization as a trait favoring the evolution of alternative reproductive tactics and morphs among teleosts because it "makes it difficult for male fishes to monopolize access to fertilizable eggs." The latter leads to males trying to "control preferential access to fertilizable eggs," as exemplified by the nest-building and vocal courtship tactic of type I males who alone guard substrate-attached eggs. (Egg-guarding would also incur additional costs to a type I male tactic.) Intense competition among males for nest sites, and thus access to a female's eggs, would then be considered a major selective force favoring the adoption of alternative type II-like male reproductive tactics.

Usually, when theorists consider the effects of evolution on an animal's physical traits, they consider body size or color, which in fact do differ between the reproductive morphs of the midshipman. My colleagues and I have considered how evolution also affects the neural substrates of display behaviors, which are also part of an animal's life history. Our studies of alternative phenotypes in midshipman fish show that sexuality for each reproductive morph or for that matter, each individual, can be defined by developmental, sexual maturity-dependent trade-offs between suites of species-typical traits. As we have seen, these trade-offs shape the brain and its behavioral sex, and provide an important link between neurobiology and behavioral ecology.
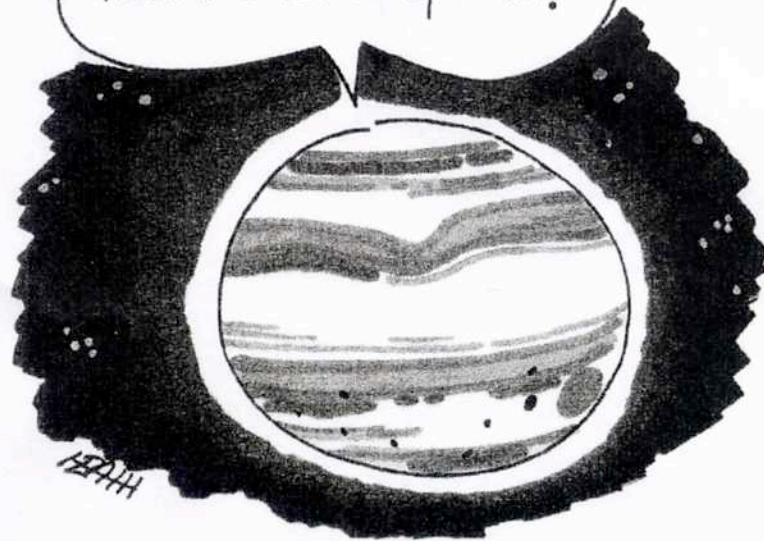
Acknowledgments

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