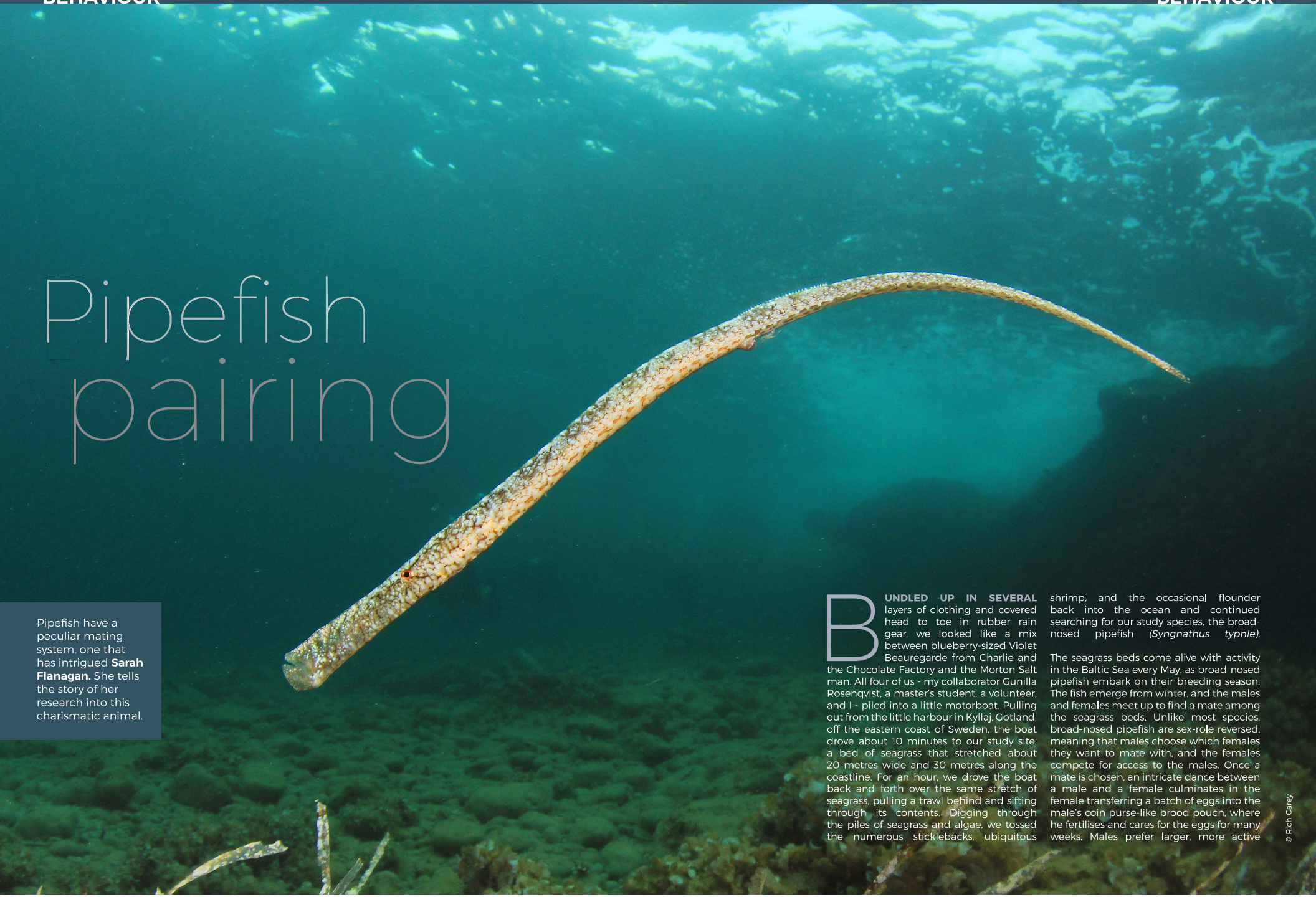
BEHAVIOUR

Pipefish pairing

Pipefish have a peculiar mating system, one that has intrigued **Sarah Flanagan.** She tells the story of her research into this charismatic animal.



BUNDLED UP IN SEVERAL layers of clothing and covered head to toe in rubber rain gear, we looked like a mix between blueberry-sized Violet Beauregarde from Charlie and the Chocolate Factory and the Morton Salt man. All four of us - my collaborator Gunilla Rosenqvist, a master's student, a volunteer, and I - piled into a little motorboat. Pulling out from the little harbour in Kyllaj, Gotland, off the eastern coast of Sweden, the boat drove about 10 minutes to our study site: a bed of seagrass that stretched about 20 metres wide and 30 metres along the coastline. For an hour, we drove the boat back and forth over the same stretch of seagrass, pulling a trawl behind and sifting through its contents. Digging through the piles of seagrass and algae, we tossed the numerous sticklebacks, ubiquitous shrimp, and the occasional flounder back into the ocean and continued searching for our study species, the broad-nosed pipefish *(Syngnathus typhle)*.

The seagrass beds come alive with activity in the Baltic Sea every May, as broad-nosed pipefish embark on their breeding season. The fish emerge from winter, and the males and females meet up to find a mate among the seagrass beds. Unlike most species, broad-nosed pipefish are sex-role reversed, meaning that males choose which females they want to mate with, and the females compete for access to the males. Once a mate is chosen, an intricate dance between a male and a female culminates in the female transferring a batch of eggs into the male's coin purse-like brood pouch, where he fertilises and cares for the eggs for many weeks. Males prefer larger, more active

BEHAVIOUR

Left: Sarah and Gunilla Rosenqvist looking for pipefish in a bucket full of seagrass and fish.

females, who are generally believed to produce better offspring. But the mating system is not as straightforward as it seems. For years, researchers studying the broad-nosed pipefish have observed that only large males are able to mate early in the season, suggesting that male quality may be shaping the mating dynamics of these fish.

That is why we braved the brisk May air and plunged our hands into the frigid waters of the Baltic Sea: to try to support anecdotal evidence with quantitative data. I wanted to find out how important male quality is in shaping the patterns of mating in broad-nosed pipefish. To do that, I needed to measure the fish early in the breeding season. Pulling them out of the jumble that came from the trawl wasn't enough, though. So after about an hour of sifting through seagrass, we rinsed out the net and headed back to shore. All of the pipefish we had caught were secured safely in buckets with bubblers, and we separated the males and females so a non-pregnant male couldn't become pregnant on the boat. Back on shore, we counted the fish as we transferred them into coolers in the back of the car, and then drove to the field station where we would be tagging and measuring them.

Tagging pipefish is a bit like giving them a quick tattoo. We inject coloured silicone just beneath the skin of the fish. I had four colours to work with, and each fish got three markings on either their left or right side. In the car, as we drove to the field station, I assigned each fish a colour code that would serve as their tag so that I would know what colours to prepare as soon as we got to the field station. In the laboratory at the field station, we lined up the fish in buckets and then pulled each fish out to anesthetise it in clove oil and inject the coloured tag. Once completed, we quickly measured the fish and took photographs before returning them to a bucket to recover from the anaesthetic, and then back to the seagrass bed where they were caught.

Nearly every day for a month, I put on my Violet Beauregarde suit and went out in the boat with my collaborator and my helpers. As the weather improved, and I got to shed some of my layers, we saw the average size of pregnant males decrease – the larger males had indeed been the first to get pregnant, but by the end of the first month of the breeding season, the smaller males had started becoming pregnant as well.

But size is not the only measure of quality. In fish, it's generally

BEHAVIOUR

"The mating system is not as straightforward as it seems"

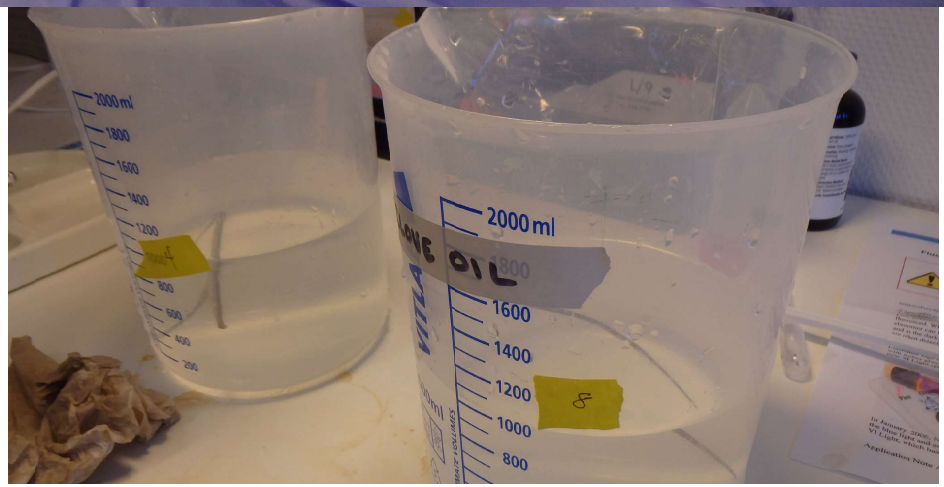
better to be fatter - the more fat you have, the better condition you are in. So once we were catching about equal numbers of pregnant and non-pregnant males, I collected a sample of females, pregnant males, and non-pregnant males from the population and measured their body fat. To do this, I dissected each fish to remove the stomach (because I didn't want to measure the fat content of their last meal), and dried the carcasses in an oven for a couple of days to remove the water weight. Then I weighed each dried fish and put it into a substance called petroleum ether, which sucks the fat molecules known as lipids out of the tissue. For several days I replaced the lipid-filled petroleum ether with new petroleum ether until all of the lipids were removed from the tissue, and then I dried and weighed the fish again. The difference in weight before and after the removal of lipids was my estimate of how much fat they contained.

Because the sample was taken during the first month of the breeding season, I knew the pregnant males in my sample were early-breeders. Those early-breeding pregnant males had more fat stores than non-pregnant males, and the larger pregnant males with more fat stores carried heavier embryos. Heavier embryos in pipefish generally survive better and result in larger offspring, which in turn do a better job avoiding predation. What this suggests is that not only does condition play a role in whether males are pregnant or not, but male condition also may influence the success of the brood. Therefore, male quality also plays a role in the quality of his offspring – it's not all down to the female.

Sander van Leeuwen tagging a pipefish using coloured silicone in the laboratory.

To attempt to pry apart the impact of male quality and female quality on the success of a brood, I paired large and small males with females in the laboratory. We found that larger females produced heavier embryos, regardless of the size of the males. So large females produce better clutches. These results help paint a picture of the complicated dynamics of the broad-nosed

BEHAVIOUR



Top: A male pipefish being held by researchers. It is pregnant - its pouch full of embryos. Bottom: Beakers of sea water and clove oil for anesthetizing the fish

pipefish mating season, which depends on the timing of reproduction and the quality of both males and females.

Early in the season, the large males are in the best condition and are the first to breed. Because there are fewer males competing for all of the females, it is likely that the early-breeders get to choose the most attractive, largest females. Therefore, those early males have larger clutches with offspring that are more likely to survive because they mated with the best females. Males who are in the best condition have increased reproductive success, simply because they are the first individuals able to mate in the population.

All of these results are consistent with the hypothesis for how sexual selection works in species with equal numbers of males and females originally proposed by Darwin in The Descent of Man. Darwin proposed that in a bird species where males compete for females, the first females to breed will be those in better condition, and they will mate with the highest-quality males. Pairs of good-condition females and desirable males would then have the best offspring. In pipefish, these roles are reversed and the sex ratios are somewhat skewed toward males, but the patterns I observed in the seagrass beds in Sweden indicate that Darwin's prediction for birds possibly applies to pipefish, as well.

Peering down at the seagrass from the side of the boat, you can't spot the pipefish living out their dynamic lives. But beneath the boat they are engaging in their complicated, sex-role reversed, mating rituals. The fish's evolutionary success doesn't just depend on whether they survive the winter and can find a mate before becoming a snack for a larger fish – it also depends on the intersecting effects of timing of reproduction, individual quality, and attractiveness, which determine how many offspring they produce and whether those offspring survive. My time spent on the boat and in the lab in Sweden helped elucidate some of the complexities in broad-nosed pipefish, but all animals likely lead similarly complex lives. So the next time you observe animals in the midst of their breeding season, remember that mating is often about more than just preferences and competition – and think of researchers putting on their Violet Beauregarde suits just to try to understand the interacting factors influencing reproductive success.

Flanagan, S. P., Rosenqvist, G., & Jones, A. G. (2017). Mate quality and the temporal dynamics of breeding in a sex-role-reversed pipefish, S. typhle. Behavioral Ecology and Sociobiology, 71(1), 28.