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Gamete Release and Spawning Behavior in Broadcast Spawning Marine Invertebrates

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INTRODUCTION

The release of eggs and sperm into the environment for external fertilization is common (Giese & Kanatani 1987) and thought to be the ancestral mating strategy (Wray 1995). Most phyla have at least some species that release sperm into the environment (free spawning or spermcasting) and a majority have representatives that also release eggs (broadcast spawning) for external fertilization (Levitan 1998a).

A common feature of broadcast spawning taxa is a lack of sexually dimorphic adult characters (Strathmann 1990; Levitan 1998a). The rare instances of adult morphological differences among sexes occur in pair-spawning species and include body size differences or specialized appendages for clasping females (Levitan 1998a). The general absence of sexual dimorphism has been used as evidence that broadcast spawning invertebrates are not under sexual selection (Darwin 1871).

In contrast to this view on the nature of sexual selection based on sexual dimorphism, recent studies documenting the rapid evolution of gamete recognition proteins in external fertilizing species have invoked sexual selection and sexual conflict as the driving force of positive selection (Palumbi 1999; Swanson & Vacquier 2002; Haygood 2004). Only recently have data been collected on the patterns of reproductive variance and the intensity of sexual selection in male and female broadcast spawners (Levitan 2004, 2005a, b; Levitan & Ferrell 2006; Levitan 2008). The results suggest that sexual selection can be intense in these species and that the nature of sexual selection is dependent on the distribution and abundance of individuals.

Given the nature of external fertilization, where the competition among individuals for mates is played out among gametes in the water column, it is not surprising that the primary sexual characteristics targeted by selection are associated with the spawning behaviors that mediate gamete competition and the traits of the gametes themselves. The influence of sperm availability and sexual selection on gamete traits has already received some attention (Levitan 1998b, 2006). Below we briefly summarize the relationship between gamete traits and fertilization and direct readers to the relevant literature. The remainder of the chapter is focused on the less studied question of how sexual selection might influence the evolution of the duration, release rate, and timing of gamete release. Because these features determine the size, shape, and duration of an individual's gamete distribution, it defines the extent to which one individual can interact with mates and mate competitors.

Gamete Traits

There is emerging evidence that selection caused by too few or too many sperm might have influenced the evolution of both sperm and eggs (Levitan 2002a;

Marshall & Keough 2003; Levitan & Ferrell 2006). Gamete traits that do best under sperm-limited conditions are those that either enhance the likelihood of sperm–egg collisions or increase the probability of fertilization given a collision. Higher rates of sperm–egg collisions can be a result of increasing egg cell size (Levitan 1993, 1996; Marshall & Keough 2003), associated structures that might capture sperm (Farley & Levitan 2001; Podolsky 2001, 2002), and chemical sperm attractants (Miller 1985; Riffell et al. 2004). Increasing the likelihood of fertilization can also be influenced by the properties of the egg surface that determine the fraction of sperm collisions that result in fertilization (Vogel et al. 1982; Levitan 1993). A potential cost to producing eggs too easy to fertilize can be an increase in the risk of heterospecific fertilizations (Levitan 2002b) or developmental failure caused by multiple sperm fusing with the egg surface (Styan 1998; Franke et al. 2002; Levitan 2004; Levitan et al. 2007). Eggs presenting a smaller sperm target, or a more restricted set of acceptable sperm, appear able to successfully block excess sperm (Levitan et al. 2007).

Sperm may also be under selection based on different levels of sperm availability. Sperm limited conditions may select for longer-lived sperm, as sperm must be viable for longer periods before encountering eggs, while sperm competitive conditions may select for more rapid sperm as unfertilized eggs are an ephemeral resource (Levitan 1993, 2000; Kupriyanova & Havenhand 2002). Empirical and theoretical examinations of how egg size might influence collision rates have caused for a reexamination of the evolution of anisogamy (eggs and sperm) from an isogamous condition (Levitan 1996; Dusenbery 2000; Randerson & Hurst 2001; Bulmer & Parker 2002; Podolsky 2004; Bode & Marshall 2007).

Spawning Behavior

Because of the difficulty associated with predicting the timing and location of spawning for most broadcast spawning taxa, there is a paucity of information on the timing (relative to mates), spawning duration (length of time gametes are released), and frequency (number of times an individual spawns in a season, or reproductive bout) of gamete release. Much of what is known is based on scattered reports of spawning behavior in nature or in the laboratory and what can be gleaned by experimentally inducing individuals to spawn via chemical or temperature stimulants. In this chapter, we are interested in how individuals parcel out their gametes during release and the effect this may have on male–female differences in time of spawning. What are the advantages and disadvantages of blasting out gametes for a short duration or dribbling out gametes for longer intervals? Does the optimal strategy depend on mate or competitor densities or environmental factors such as water flow? Do males and females pursue different spawning strategies?

Most theoretical treatments of the mechanics of spawning assume that individuals spawn as a plume. Plumes are a steady release of gametes that, in principle, establish a constant gradient of gamete concentration from the point of release. However, when gamete release is of short duration, a constant gradient may not be established and a time-dependent concentration gradient is established as gametes disperse from a source. Acknowledging that gamete release is an ephemeral process and that gamete concentration gradients have an ebb and flow is a necessary prerequisite for examining the consequences of different spawning strategies.

In this chapter we first survey different patterns of gamete release among taxa. We then discuss experimental manipulations of gamete release to examine how different release strategies influence male and female reproductive success under a variety of demographic conditions. Then, we introduce a model of turbulent diffusion from a point source that can be used to estimate gamete concentrations for different spawning durations. We expand this model to a two-dimensional population to explore how advection, male density, and male spawning duration affect the time when females choose to initiate spawning. Then, using comparative data from the literature, we examine the evidence for predictions from the model. Finally, we discuss how the effectiveness of these strategies may vary with the level of sperm competition, population density, and ambient water flow.

PATTERNS OF GAMETE RELEASE

Because our ability to predict the timing and location of spawning in marine populations is limited, and spawning is often nocturnal, many observations of natural spawning events are on lone individuals or a small portion of the population. In particular, although it is common to report the time

of spawning in relation to some environmental variable (i.e., sunset or day in a lunar cycle), spawning duration is not commonly measured. For this section, we reviewed the literature in order to gain insight into the different strategies for gamete release in broadcast spawners.

We concentrate on broadcast spawning species, in which sperm and eggs are released into the water column. Spermcasting species are organisms in which males release sperm into the surrounding water, but females that retain eggs (e.g., some sponges—Reiswig 1970; byrozoans and ascidians—Yund & McCartney 1994; gorgonians—Lasker 2006) may have a very different dynamic (Bishop 1998). Females that retain eggs on or inside their surface are able to integrate sperm concentration in the seawater over the life of the egg. High fertilization rates have been observed as a result of this strategy (Phillippi et al. 2004; Lasker 2006). The dynamics of broadcast spawning species are different because the concentrations of sperm and eggs become diluted in the water column, and fertilization rates will decrease with decreasing concentrations. Therefore, the rate and timing of gamete release will have a large effect on fertilization rates of broadcast spawners.

The physiological act of spawning places gametes into the environment. The dispersal and dilution of those gametes can be influenced by the viscosity of the spawned material, adult morphology and posture, and the interaction of these factors with water flow. In polychaetes, sperm appear to advect away from adults in a plume, while eggs can form strings or clumps that retain on the female for seconds to minutes before being released (Thomas 1994a). Thomas (1994b) has suggested that adults may increase the viscosity of spawned material when flow and turbulence are high to reduce the rapid dilution of gametes. Enhanced residence times of eggs on the female's surface would allow eggs to sample the water column for sperm passing by. Under some conditions this might lead to higher levels of fertilization compared to eggs released into the water column that drift with a particular parcel of water.

Adults can also influence how flow interacts with gamete dispersal through movement patterns and posture. Many species climb objects prior to spawning or assume a posture that places their gonopores into the water column (Pearse 1979; McEuen 1988; Hendler 1991; Babcock et al. 1992; Minchin 1992; Stekoll & Shirley 1993). Releasing gametes under the higher flow conditions above the surface boundary layer would increase the rate at which they would be advected away from adults (Yund & Meidel 2003). This suggests that not all species behave in a way that would enhance gamete retention; in some situations gamete dispersal may be advantageous.

Differences in viscosity, behavior, morphology and habitat may explain why in some taxa, gametes seem to disperse immediately into the water column, while in other taxa they may reside for short periods before being advected away. Observations of gametes being released directly into the water column include cnidarians and echinoderms (Minchin 1992; van Veghel 1994; Himmelman et al. 2008). In some cases, notably temperate sea urchins, eggs and sperm pool on the aboral surface for several seconds to minutes before being advected off the adult surface (Minchin 1992; Levitan 2002a; Himmelman et al. 2008). Sea urchins may retain gametes longer because their spines might create a boundary layer around the adults; however, the tropical sea urchin *Diadema antillarum*, which has extremely long spines, spawns gametes directly into the water column (DRL personal observation), suggesting that viscosity or expulsion rate may also influence dispersal rate. The temperate sea urchins that often retain gametes also live in wave swept environments, and gametes appear to lift off at greater rates during the change in water direction associated with wave action (DRL personal observation), which is when flow is accelerating and presumably highly turbulent. Laboratory experiments of gamete fertilization of sea urchins in mild to moderate unidirectional flow, where changes in velocity are eliminated, indicate that eggs are slowly released off of females for several hours and that a proportion of those eggs are fertilized during this period (Yund & Meidel 2003). But because individuals can spawn for several hours (Levitan 2002a), these experiments cannot distinguish between long residence times (the length of time a gamete remains on the surface) and long spawning times.

The spectrum of gamete release observed in a variety of taxa ranges from organisms that release their gametes in a near instantaneous release ("puff"), to organisms that spurt gamete batches or bundles over a period of time ("pulse"), to organisms that continuously release gametes from several minutes to over nearly an hour or longer ("plume"). Because measurements of spawning duration are not the main focus of most studies, the observations we cite

are based on natural spawning in the field or laboratory and usually only for a few individuals (table 6.1).

A majority of observations are from organisms that release gametes continuously. Continuous spawners can release gametes for just a few seconds to several minutes to an hour or more (table 6.1, figure 6.1a, b). The advantage of releasing gametes for an extended period of time is that if synchrony in the population is low, the probability of finding a mate is increased. The cost of this behavior is a reduction in the concentration of gametes

TABLE 6.1 Spawning observations of marine invertebrate species that release gametes continuously (as a puff or plume) or in pulses. If the sexes exhibited different strategies, or only one sex was observed spawning, it is noted below

Species	Taxa (Phylum: Class: Order)	Spawning Behavior	Reference
Dryodora glandiformis, Bolinopsis vitrea, Pleurobrachia bachei	Ctenophora: Tentaculata	***Pulse:*** Sperm are released in bursts over a 5 minute period, followed by bursts of egg release over 5–10 minutes (hermaphrodite).	Pianka 1974
Macrorynchia philippina	Cnidaria: Plumulariida: Aglaopheniidae	***Both:*** Spawning of swimming medusiods lasted 1–2 minutes. Males released sperm continuously and females released batches of 1–4 eggs with each contraction.	Bourmaud & Gravier-Bonnet 2004
Heteractis magnifica	Cnidaria: Anthozoa: Actiniaria	***Pulse:*** One female was observed releasing 1 to 10 eggs at a time over a period of 1 hour.	Babcock et al. 1992
Montastraea annularis complex	Cnidaria: Anthozoa: Scleractinia	***Continuous:*** Synchronous release of buoyant egg-sperm bundles across the entire coral head under a minute.	Szmant 1986; Levitan et al. 2004
Montastraea cavernosa	Cnidaria: Anthozoa: Scleractinia	***Pulse:*** Sperm expelled from male colonies as repeated plumes; female colonies rapidly released eggs.	Gittings et al. 1992
Acropora aspera group	Cnidaria: Anthozoa: Scleractinia	***Continuous:*** Colonies spawned over a 15–20 minute period.	Van Oppen et al. 2002
Stephanocoenia intersepta	Cnidaria: Anthozoa: Scleractinia	***Continuous:*** Male colonies release sperm over a period of 4–5 minutes, and females release eggs over 2–3 minutes.	Vize et al. 2005
Diploria strigosa	Cnidaria: Anthozoa: Scleractinia	***Continuous:*** Eggs released over an 8 hour period, sperm release not noted.	Wyers et al. 1991
Pseudoplexaura porosa	Cnidaria: Anthozoa: Gorgonacea	***Continuous:*** Males spawn for 60–90 minutes and females spawn for 25–40 minutes.	Coma & Lasker 1997
Phragmatopoma californica	Annelida: Polychaeta	***Pulse:*** Males release a series (1–10) of sperm clouds or clumps into the water column over a 1–15 second period. ***Continuous:*** A female released eggs for 193 seconds.	Thomas 1994b
Spirobranchus giganteus	Annelida: Polychaeta	***Continuous:*** Males and females spawned for approximately 15 minutes.	Babcock et al. 1992
Aspidosiphon fischeri (formerly: *Paraspidosiphon fischeri*)	Sipuncula: Phascolosomatidea: Aspidosiphoniformes	***Pulse:*** A male spawned seven times in the laboratory over a period of 40 minutes (females not noted).	Rice 1975

TABLE 6.1 *(Cont.)*

Species	Taxa (Phylum: Class: Order)	Spawning Behavior	Reference
Phascolion cryptum (formerly: *Phascolion cryptus*)	Sipuncula: Sipunculidea: Golfingiiformes	***Pulse:*** Released short intermittent spurts of sperm over 15 minutes (females not noted).	Rice 1975
Glottidia spp. and *Terebratalia* spp.	Brachiopoda	***Continuous:*** Eggs were shed for an hour to several hours (males not noted).	Long & Stricker 1991
Mopalia lignosa	Mollusca: Polyplacophora: Neoloricata	***Pulse:*** Males observed to release sperm in spurts lasting 3–5 minutes at 5–15 minute intervals. ***Continuous:*** Females released a steady stream of eggs.	Pearse 1979
Lepidochitona cinereus	Mollusca: Polyplacophora: Neoloricata	***Continuous:*** Females released eggs over about 2.5 hours (males not noted).	Pearse 1979
Crassostrea spp.	Mollusca: Bivalvia: Ostreoida	***Pulse:*** In females eggs are expelled in batches by contractions of adductor muscle (males not noted).	Andrews 1979
Hyotissa hyotis, Chama spp., *Arca* spp.	Mollusca: Bivalvia	***Pulse:*** Gametes shed intermittently over a period of 15 minutes.	Babcock et al. 1992
Tridacna gigas	Mollusca: Bivalvia: Veneroida	***Pulse:*** Males released sperm in contractions spaced 2–5 min apart, lasting for 1–2 hours. Female spawning not noted.	(Babcock et al. 1992)
Acanthaster planci	Echinodermata: Asteroida: Valvatida	***Continuous:*** Spawning in both sexes lasted for about 30 minutes to an hour.	Babcock et al. 1992; Babcock et al. 1994
Linkia laevigata	Echinodermata: Asteroida: Valvatida	***Continuous:*** Gamete release lasted 15–30 minutes.	Babcock et al. 1992
Amphioplus abditus	Echinodermata: Ophiuroidea: Ophiurida	***Pulse:*** Release of gametes was intermittent over 20 minutes.	Hendler 1991
Gorgonocephalus eucnemis	Echinodermata: Ophiuroidea: Ophiurida	***Continuous:*** Spawned for 1.5 hours.	Hendler 1991
Ophiothrix angulata	Echinodermata: Ophiuroidea: Ophiurida	***Continuous:*** In females all eggs were shed "explosively" in one session.	Hendler 1991
Ophiothrix orstedii	Echinodermata: Ophiuroidea: Ophiurida	***Pulse:*** Gametes ejected in 5 second bursts alternating with 30 second intervals of repose.	Hendler 1991
Ophiura robusta	Echinodermata: Ophiuroidea: Ophiurida	***Continuous:*** A mass spawn lasted 30 minutes.	Himmelman et al. 2008
Ophiopholis aculeata	Echinodermata: Ophiuroidea: Ophiurida	***Continuous:*** A mass spawn lasted 20 minutes.	Himmelman et al. 2008
Strongylocentrotus droebachiensis	Echinodermata: Echinoidea: Echinoida	***Continuous:*** Individuals were observed to spawn for at least one hour.	Himmelman et al. 2008
Strongylocentrotus fransicanus	Echinodermata: Echinoidea: Echinoida	***Continuous:*** Males were observed to spawn for 140 minutes and females for 60 minutes.	Levitan 2002a
Diadema antillarum	Echinodermata: Echinoidea: Echinoida	***Continuous:*** Urchins were observed to spawn for at least 2 hours.	Randall et al. 1964

Continued

TABLE 6.1 *(Cont.)*

Species	Taxa (Phylum: Class: Order)	Spawning Behavior	Reference
Cucumaria lubrica	Echinodermata: Holothuroidea: Dendrochirotida	***Continuous:*** In males, sperm exit the gonopore in bundles over a period of 2 or more hours. Females began to spawn 2–7 hours after males, and released eggs for 1–4 hours.	McEuen 1988
Cucumaria miniata	Echinodermata: Holothuroidea: Dendrochirotida	***Continuous:*** Males released sperm for 3–6 hours. ***Pulse:*** Females released egg packets every 4–10 minutes, over a period of 0.75–4 hours.	McEuen 1988
Psolus chitonoides	Echinodermata: Holothuroidea: Dendrochirotida	***Continuous:*** Males spawned for 1–3 hours, females spawned for 1–1.5 hours.	McEuen 1988
Pentamera populifera	Echinodermata: Holothuroidea: Dendrochirotida	***Pulse:*** Males emitted sperm in pulses lasting 2.75–3 minutes, as many as 5 pulses were visible. ***Continuous:*** Females released eggs for 1–2 hours.	McEuen 1988
Molpadia intermedia	Echinodermata: Holothuroidea: Molpadiida	***Pulse:*** In males, 1–2 second long puffs of sperm spurted out at intervals of 0.7–6.5 min. ***Continuous:*** A female was observed to release all eggs in an explosive burst.	McEuen 1988
Isostichopus fuscus	Echinodermata: Holothuroidea: Aspidochirotida	***Continuous:*** Females released gametes on average for 48 min (± 18 min St. Err.) and males for 62 min (± 18 min St. Err.).	Mercier et al. 2007
Stephanometra spp.	Echinodermata: Crinoidea: Comatulida	***Pulse:*** Several short bursts lasting 2–3 seconds.	Babcock et al. 1992
Lamprometra klunzingeri	Echinodermata: Crinoidea: Comatulida	***Continuous:*** Spawning for both sexes lasts only about 25 seconds.	Holland 1991
Oxycomanthus japonicus	Echinodermata: Crinoidea: Comatulida	***Continuous:*** Females spawned within 5 minutes.	Holland 1991

(compared to if all were released at once). This might influence reproductive success in females if optimal sperm concentrations are not reached and in males if high concentrations are needed to assure paternity during male competition. The advantage of releasing gametes in an explosive burst, is that gamete concentrations are not reduced (compared to if they were released over a period of time). However, this requires high synchrony with mates because gamete concentrations are more ephemeral.

Broadcasting spawning corals are an interesting case because they are hermaphroditic, and package gametes into egg–sperm bundles. In the *Montastraea annularis* complex, these bundles are released almost simultaneously across the entire coral head, where they float to the surface and break apart for fertilization (Szmant 1986; Levitan et al. 2004). Since self-fertilization is not evident in these species, this strategy ensures initially high concentrations of both types of gametes, but apparently is only successful if population synchrony is high (Levitan et al. 2004). Not surprisingly, spawning in these coral populations is highly predictable and can be calculated from lunar calendars and sunset times (Szmant 1986; Levitan et al. 2004).

Some animals may spawn for a length of time, but not do so continuously as in pluming organisms. For example, some invertebrates release gametes for several minutes, stop for a period of time,

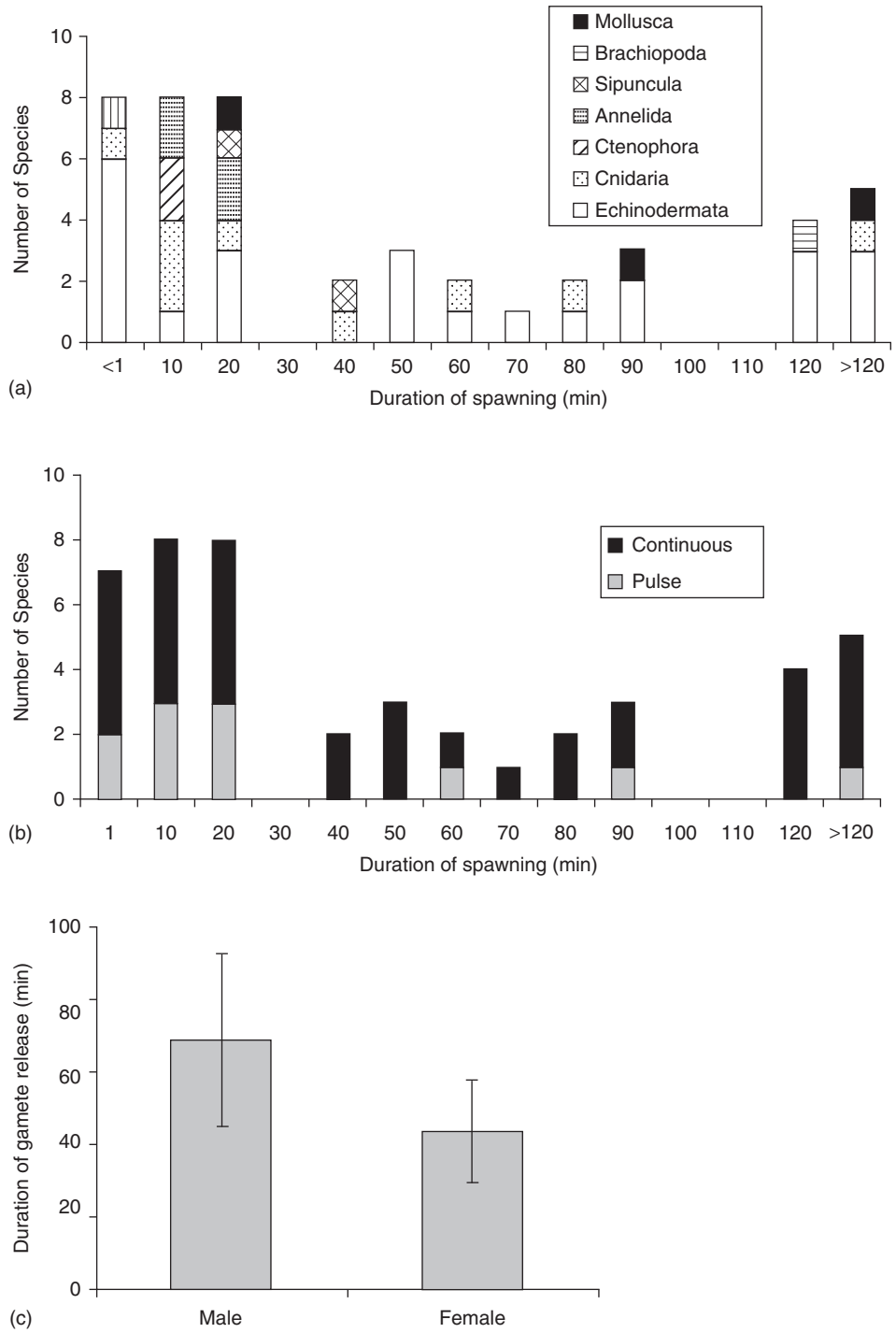

FIGURE 6.1 Results from a survey on gamete release observations in nature. Males and females were counted as separate within a species. (a) Histogram of spawning duration organized taxonomically. (b) Same histogram organized by type of gamete release (continuous or in pulses). (c) Average spawning duration for both males and females within a species, from studies in which both sexes were reported. Means are significantly different based on a paired *t*-test.

and then start releasing again (table 6.1). This may last for an hour or more. Similarly, other invertebrates may release gametes in quick, successive spurts that last anywhere from less than a second to over a half an hour. Whether the difference between individuals releasing the same number of gametes continuously or in pulses is related to fertilization success or is a result of physiological constraints has yet to be investigated.

We used the studies cited in table 6.1 to examine whether duration of gamete release exhibited taxonomic patterns, release patterns, or sex differences. For this analysis, males and females were counted as separately within a species if reported, and the midpoint was used if a range of spawning duration was reported. Only species for which total duration was reported were used. A wide range of behaviors are noted, even with a taxonomic group (e.g., Echinodermata, figure 6.1a). In order to examine whether there was a difference between continuous and pulsing spawners, we plotted the duration of gamete release against the number of species (figure 6.1b). On average, pulsers (n = 11, mean = 32.5 ± 13.9 min) tended to have a shorter spawning duration than organisms that spawn continuously (n = 34, mean = 67.1 ± 16.2 min, means marginally non-significant by t-test assuming unequal variances, p = 0.057). Within a species, we tested if there were significant differences between males and females in their spawning duration (n = 13, data was only used if male and female duration was observed in the same study). While the duration of spawning between males and females within a species was highly correlated (Pearson's correlation coefficient = 0.94), females spawned for a significantly less duration than males (figure 6.1c, one-tailed paired-t-test, t-stat = 2.17, p = 0.025). This supports the observation that females tend to start spawning after males and both sexes finish at the same time (Hamel & Mercier 1996; Levitan 2002a). Overall, observations of spawning durations in nature are spread throughout a wide range of times, but there may be considerable bias toward shorter times because they are easier to quantify. Notably, there appears to be no taxonomic pattern, which suggests that phylogenetic constraints do not limit these behaviors and that contemporary selective pressures (perhaps associated with demography or water flow characteristics) might drive spawning durations.

To date there is not enough data to make general conclusions about how spawning duration may vary within and between individuals. For instance, individuals may increase the rate of spawning in response to cues from conspecifics, which has been occasionally observed (Levitan 1998a; Himmelman et al. 2008). The duration of gamete release, along with the advective environment and the number of gametes released per unit time will determine the size and extent of the gamete cloud (Denny & Shibata 1989), and thus interactions with conspecifics of the opposite sex. In the next section, we outline experimental data that examines the consequences of variation in spawning times. Then, we use a model to explore how different spawning durations affect the distribution of gametes, and how this affects female choice in when to spawn.

EXPERIMENTAL MANIPULATIONS

Sea urchins provide a good model for mechanistic studies of gamete interactions because spawning is easy to manipulate, by KCl injection, and gamete performance can then be examined through controlled artificial releases in either the laboratory or field. In addition, adults can be manipulated to spawn at different times in the field, allowing examination of the costs and benefits of synchronous and asynchronous spawning behavior.

Although artificial induction of spawning removes the natural decision over when and where to spawn, there is some evidence that induced spawning events can closely mimic natural spawning events in terms of gamete release, aggregative behavior and fertilization rates in at least some species. Experimentally induced spawning events in which all individuals within a 5 × 5 meter area were injected with KCl, and immediately placed back in their original position had the same fertilization rates and nearest neighbor distances compared to naturally spawning sea urchins at those same densities (Levitan 2002a). This indicates, for at least some sea urchin species, that gamete release rates, gamete quality, and adult aggregative behavior are not markedly different during natural and induced spawning events.

Syringe Release Experiments

Gametes from the sea urchin *Strongylocentrotus franciscanus* were collected from adults and released via syringes to examine how subtle differences in the timing and position of gamete release influenced

male and female fertilization success in and out of sperm competition (figure 6.2). Male success in the presence of competition was determined using microsatellite markers to assign paternity. Eggs and sperm were released in a 5-second burst at the same release rate as natural gamete release (Levitan 2005b). Sperm from either one or two males were released before and/or after egg release in 20-second intervals to examine the consequences of spawning early or late in the presence or absence of a competing male.

In the first set of treatments, all gametes were released into the center of the same parcel of seawater (which drifted downstream with the natural current), such that all subsequent releases were in the center of the gamete cloud of the initial gamete release. This mimicked individuals in a downstream linear arrangement, where gametes from one individual would pass over another individual, who would release gametes into that gamete cloud and then the pooled gamete cloud would potentially pass over a third individual for a similar release. The time differences between gamete releases (20 seconds) could also be viewed as the distance downstream between individuals; the distance gametes might drift and disperse in 20 seconds.

Two sets of treatments released gametes in the same relative position (figure 6.2a,b). Over both treatments, female success did not depend on whether male or female gametes were released first, but a higher proportion of eggs were fertilized when two males competed, because the total sperm released doubled (figures 6.2a,b, 6.3a). In the first

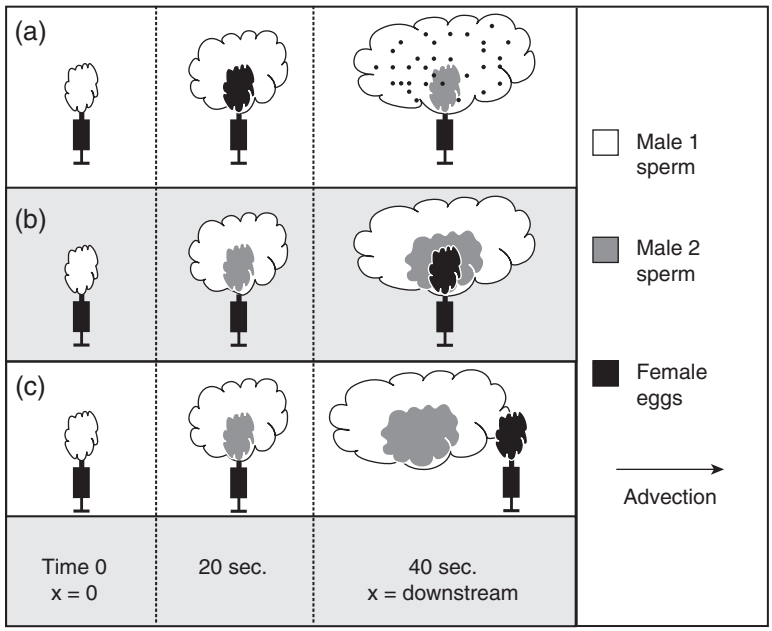

FIGURE 6.2 Schematic of the three experiments that examined the reproductive success of different sex-specific spawning behaviors under male competition (Levitan 2005b). The order of gamete release is presented from left to right, color-coded by male 1 (white), male 2 (grey), and the female (black). Gamete clouds were followed downstream as they dispersed to mimic a spawning event. (a) Spawning in same location with one male first: sperm from male 1 is released 20 seconds prior to egg release and male 2 was released 20 seconds post-egg release. Results from this experiment are presented in figure 6.3b. (b) Spawning in same location with both males first: sperm from male 1 is released 40 seconds prior to egg release, sperm from male 2 is released 20 seconds prior to egg release, and eggs are released in the center of the sperm cloud. Results from this experiment are presented in figure 6.4a. (c) Spawning at different locations with both males first: sperm from male 1 is released 40 seconds before egg release, sperm from male 2 is released 20 seconds before egg release, and eggs are released 1 m from the sperm cloud. Results from this experiment are presented in figure 6.4b.

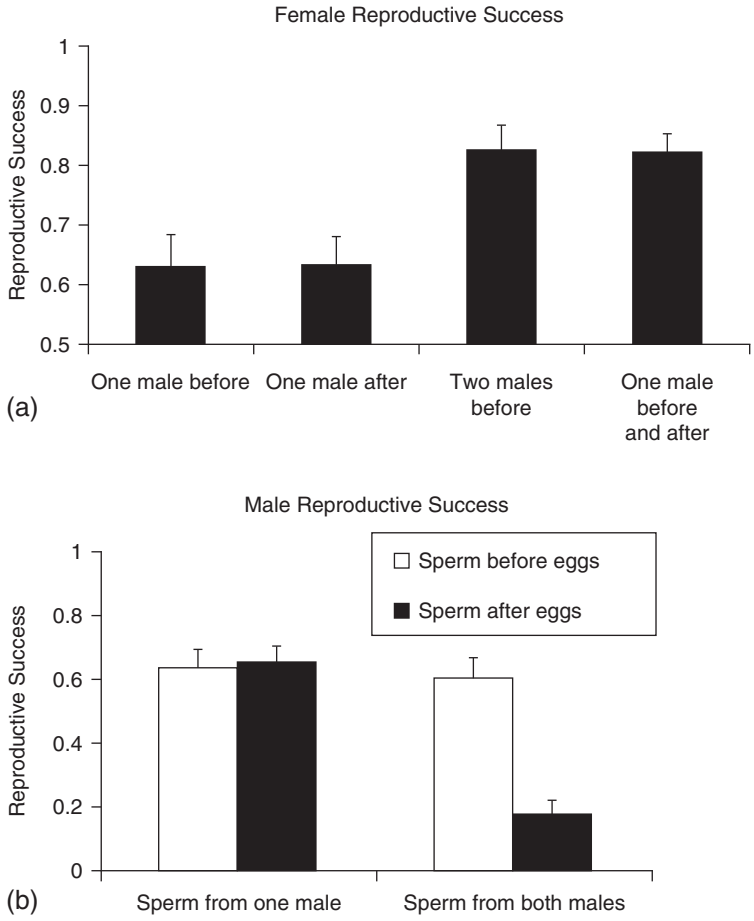

FIGURE 6.3 Male and female reproductive success as a function of synchrony and sperm competition. (a) Female reproductive success was higher when sperm from two males were released, but it was not dependent on spawning order (figure 6.2 a, b). (b) In the absence of competition, male reproductive success was the same if sperm was released before or after females. Sperm from the "one male" treatment tested each male in independent trials. In the presence of competition, the sperm released before the eggs were released garnered an advantage. For details see Levitan (2005b).

set of treatments, one male spawned before and one male after the female (figure 6.2a). When only one male released sperm, there was no difference in male success when sperm was released before or after egg release (figure 6.3b), but in competition, males that spawned after females lost to males that spawned before females (figures 6.2a, 6.3b). In the second set of treatments, both males spawned before the female (figure 6.2b). Males that spawned in closer synchrony with females (20 seconds prior to females as opposed to 40 seconds prior to females) garnered more fertilizations, especially so when in direct competition (figures 6.2b, 6.4a). This set of trials indicated that when males compete, they should be selected to spawn just prior to females, assuming that they are physically close to females.

A third set of treatments considered spatial variance, such that subsequent gamete releases were one meter from the center of the gamete cloud (figure 6.2c). These different scenarios attempted to capture the potential trade-off of a male spawning earlier before female release and producing a more diffuse gamete cloud that covered a larger spatial area compared to a later spawning male that produced a more concentrated gamete cloud with

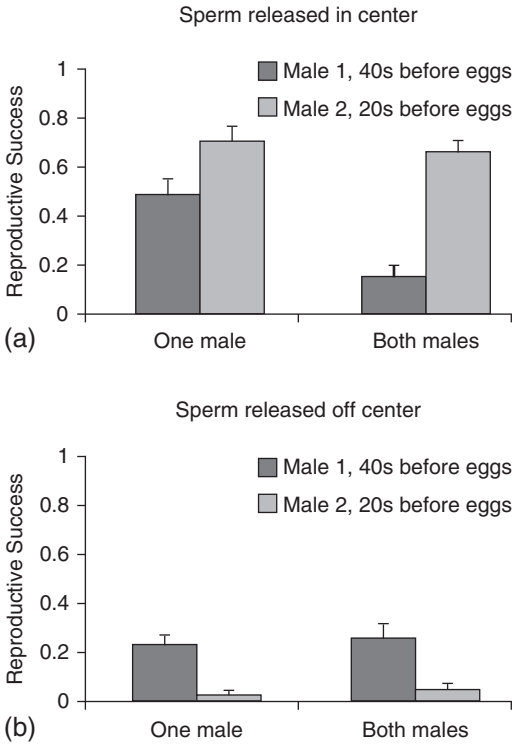

FIGURE 6.4 Male reproductive success when sperm were released 40 or 20 seconds before eggs were released. Eggs were released either (a) in the center of the sperm cloud or (b) 1 m from the center of the sperm cloud. Sperm from the "one male" treatments tested each male in independent trials. The male that released first had higher success when eggs were released away from the sperm cloud, but lower success if eggs were released in the center of the sperm cloud. For details see Levitan (2005b).

limited spatial coverage. In these trials that examined spatial variation in spawning, early spawning males had an advantage over later spawning males that were more synchronous with females. The sperm from early spawning males had the time to spread over a larger spatial area and could fertilize eggs and outcompete more synchronous males whose sperm was more concentrated over a smaller spatial area (figures 6.2c, 6.4b). These results suggest that males are selected to spawn before females, and the time difference between male and female spawning may depend on the spatial distribution of individuals and the degree of sperm competition (Levitan 2005b).

Gamete Release from Induced Spawning Experiments

The releases of gametes in the above experiments were in short puffs and the timing differences were staged at 20 second intervals. This provides a measure of the relative consequences of subtle differences in spawning times, but spawning durations in marine invertebrates can often extend for an hour or longer (table 6.1). To test the patterns noted above over longer spawning durations, individual sea urchins were induced to spawn at different intervals to determine how early or late initiation of male spawning influenced male reproductive success over a range of male to female distances and water flow (figure 6.5). Males placed in the center of the experiment were initiated to spawn 20 minutes, 10 minutes and 5 minutes before eggs were collected from spawning females. Females were placed between 0.5 and 3.5 meters from the group downstream of spawning males. Parentage of collected embryos from each female was determined from microsatellite loci and male reproductive success was examined as a function of how early he initiated spawning and the distance between the male and female (figure 6.5). The results indicated that early spawning males sired an equal number of

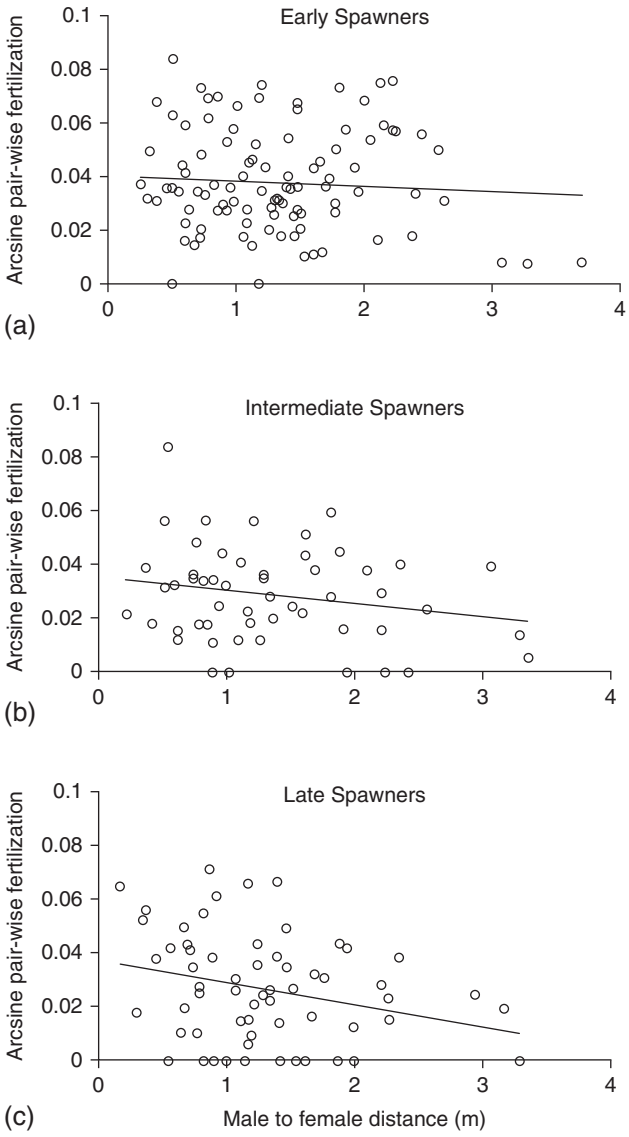

FIGURE 6.5 Fertilization success of a particular male–female pair (determined by microsatellite paternity assignment) as a function of the distance between them, for early, intermediate, and late spawning males. Significance of advection and male–female distance on fertilization success was tested with a multiple regression. (a) Early-spawning did not have a significant effect for male–female distance ($P < .05$). (b) For intermediate spawners this effect was marginally significant and (c) late-spawning males had a significant effect of male–female distance. For late spawners, greater distance between males and females resulted in lower levels of fertilization. For details see Levitan (2005b).

offspring independent of female distance, while late spawning males had decreasing paternity with female distance. This shows that early spawning males were able to more evenly cover the full spawning array than late spawning males.

These results from both short and long duration of gamete release, suggest that for sessile or sedentary organisms, there may be a benefit for males to spawn over a protracted period prior to females releasing eggs, so that sperm have already

permeated the environment before females release their eggs. Another benefit to early spawning males is that their sperm will potentially be exposed to a wider diversity of egg genotypes. Variation in sea urchin reproductive success is in part a function of intraspecific gametic compatibility (Palumbi 1999, Evans & Marshall 2005, Levitan & Ferrell 2006). Field experiments with this same species of sea urchin indicate that even in the face of wide variation in spatial positioning during spawning events, male and female reproductive success could be predicted by their gamete recognition protein genotype (Levitan & Ferrell 2006).

The cost of spawning over a protracted period is that sperm are less concentrated at the point of release, assuming an inverse relation between spawning duration and the rate of gamete release. A potential cost to spawning too early is either missing the spawning event entirely or potential loss of gamete function caused by aging. Egg longevity is on the scale of several hours (Pennington 1985), while sperm longevity is dependent on sperm concentration (Levitan et al. 1991). Sperm, while concentrated remain inactive and can survive for hours to days, particularly at cold temperatures (Chia & Bickell 1983). Once advected into the water there appears to be a linear decline in sperm half-life with dilution, such that sperm at the boundary of being dense enough to fertilize any eggs have half-lives of around 5 to 10 minutes (Levitan 1993). While spawning events can certainly last longer than 5 minutes, the residence time of a particular cohort of sperm over the spawning event, once they are advected off the males and become diluted in the water column, is likely to be fairly short (on the scales of seconds to minutes at typical flow velocities; Levitan 2002a). When advection is low, bottom topography is complex (thus retaining gametes), or populations are sparse, sperm longevity may become increasingly important. Interestingly, of the three species that inhabit this environment in British Columbia, there is a correlation between sperm longevity and average population density; the species living at lowest densities has the greatest longevity.

To date, empirical data have been focused on why males may want to spawn sooner than or in synchrony with females, based on competition with other males. This data suggests that for females, the number of spawning males can influence reproductive success and thus spawning behavior. In order to explore the timing for females to initiate spawning given particular conditions, we introduce a model of gamete dispersal when releases are of finite duration rather than a constant plume.

MODELING FEMALE CHOICE IN A MALE POPULATION

Although there is a need for continued experimental studies to further examine the costs and benefits of different spawning strategies as a function of flow, spatial scale, and adult mobility, simulation models provide another tool for making predictions and generating hypotheses. Here we introduce an advection–diffusion model to explore how female behavior is predicted to be affected by male spawning duration, male density, and advection. This model estimates how a group of particles released from a point source diffuses through time (Box 6.1). We integrate this model over the spawning duration of an organism in order to understand how plumes are established and dissipate in space and time.

The type of model that has traditionally been used to explore fertilization success in marine broadcast spawners is the time-average plume model (Denny & Shibata 1989). After gametes have been released for a sufficient period of time, the plume model describes the spatial concentration gradient of gametes as a function of distance from the spawning organism. This model is independent of time, and has been used to model organisms that release gametes over long periods of time, such as sea urchins and seastars (Babcock et al. 1994; Levitan & Young 1995; Claereboudt 1999; Meidel & Scheibling 2001; Metaxas et al. 2002; Lundquist & Botsford 2004; Lauzon-Guay & Scheibling 2007).

The plume model, however, cannot be used to describe how concentrations change when an organism initiates spawning, or when an organism ceases to spawn. It can only be used to describe concentrations once a gradient has been established. As advection decreases, we can expect that it will take a longer period of time for a constant concentration to be established at downstream locations. Therefore, the plume model is not useful for exploring the reason for sexual differences in spawning, or how varying spawning duration in males can affect downstream sperm concentrations.

In order to understand how plumes are established at a downstream mate when an organism starts spawning, we introduce a model that describes

BOX 6.1 A Model of Turbulent Diffusion from a Point Source

The equation describing the concentration of particles released from a point source can be obtained from classic texts on turbulent diffusion (Csanady 1973; Okubo 1980). The equation for two-dimensional diffusion from a point source is:

$$C(x,y,t) = \frac{Q}{(2\pi)^{1/2}\sigma_x\sigma_y}\exp\left[-\frac{(x-\alpha t)^2}{2\sigma_x^2}-\frac{y^2}{2\sigma_y^2}\right]$$

where C is the concentration at an x, y location at time t; Q is the total number of gametes released at the source; and σ is advection, which is assumed to be in the x-direction. The parameters σ_x and σ_y describe the variance in the spread of the particle cloud, which increases non-linearly with time:

$$\sigma_i = (2tD_i)^{1/2}$$

where t is time and D_i is the diffusion constant in the given direction.

This model estimates how a cloud of particles that are released from a point will diffuse through space and time. Therefore, in order to extend this model to explore different spawning durations, we integrate the point source model over every second for the duration of spawning. It is in this case essentially a plume model, but unlike the plume model it is able to describe the establishment and decay of a plume at a downstream mate as a function of time. It is therefore useful for examining the effect of different spawning durations. Since the point source model is integrated over the number of seconds an organism spawns for, the parameter Q is calculated as the rate of gamete release (the total number of gametes divided by the number of seconds an organism spawns).

The model is limited in that it assumes gametes are released independently. If gametes are held together by mucoid substances in clumps, fertilization models may be significantly underestimating fertilization success for free-spawners (Thomas 1994b). Additionally, the model assumes that the advective environment is homogeneous, and does not estimate the effect of instantaneous turbulent structures such as vortexes. Theoretical models predict that fertilization success may become enhanced if egg and sperm filaments become trapped in these instantaneous structures (Crimaldi & Browning 2004; Crimaldi et al. 2006). However, the model presented here is practical because it can be used to simulate spawning in a population of organisms, and it predicts the average concentration of gametes as a function of distance and time.

the time-dependent diffusion of a point source (Box 6.1). For the purposes of this exercise, we assume that our population is spread out on a two-dimensional plane, on which diffusion occurs. The question we use this model to address is: given a particular pattern of spawning in an upstream male population (i.e., advection, density, and spawning duration), when will females initiate spawning? Laboratory flume experiments with unidirectional flow indicate that most fertilization happens near or at the female (Yund & Meidel 2003). Therefore, the model assumes that female behavior is based on sperm concentration at the female, at the time when she can get the highest proportion of her eggs fertilized.

In the model, when sperm is saturating (above the sperm threshold for 100% fertilization), females will initiate spawning at the time this threshold is reached. Although this threshold varies within and among species and is influenced by water flow (Levitan et al. 1991; Levitan 1998b), we use a threshold of 10^5 sperm per mL, since empirical research has shown that this is the sperm concentration by which a female can have 100% of her eggs fertilized (Levitan 2002b). If sperm concentrations in a modeled reproductive bout do not reach the 10^5 threshold, (i.e., if sperm is limiting), we assume that females initiate spawning when sperm concentration has reached 90% of the maximum.

We modeled a male population of varying natural densities (1, 2, 3, 4, 6, 8, 12, 16, 24, 32, or 48 males in a 4-m^2 area) in a square area and placed a female at the downstream central edge of this male population. Stochasticity in the model results from the random placement and start time of sperm release for all males in each replicate. Organisms are assumed to be sessile and do not move while they are releasing gametes. The model was parameterized with data from sea urchins, and each male in the population had a spawning duration of 100 minutes (typical of sea urchins). The spread of spawning initiation in the male population was normally distributed, with 95% of males beginning to spawn within approximately 45 minutes, which is typical for sea urchins (figure 6.6; Levitan 2002a). Since the magnitude of advection can affect how quickly plumes get established, and therefore female spawning behavior, we modeled advections of 0.01, 0.02, and 0.05 m/s. For all trials, we kept diffusion coefficients constant at $D_x = 0.006$ and $D_y = 0.0006$. We choose these values because they are within the range of those found in marine environments (Koehl et al. 1993; K. Lotterhos unpublished data), and it is typical for diffusion in the direction of advection (x-axis) to be an order of magnitude larger (Csanady 1973). In order to explore how spawning duration in the male population affects female choice, we also modeled the same set of parameters with each male having the same gamete release rate, but a shorter spawning duration of 10 minutes.

For each set of parameters, the response variable (timing of female initiation) was averaged for at least 20 replicate runs. We report the average time of spawning initiation in the female, in relation to the average time of spawning in the male population. We would like to emphasize that a negative value of female spawning initiation does not mean that females spawn before all males, but only before the male average.

MODEL RESULTS

In all scenarios modeled, it always benefited females to spawn after at least some males had initiated spawning, since it took time for advection to deliver sperm from the males to the females. However, in the highest density scenarios, the females are predicted to begin spawning before a large proportion of males have initiated spawning, since in these situations enough males have already released sperm to fertilize 100% of a female's eggs. We define the time for a female to initiate spawning as (a) when sperm concentrations reach 10^5 sperm per mL, and a female can have 100% of her eggs fertilized, or (b) when sperm concentrations are limiting

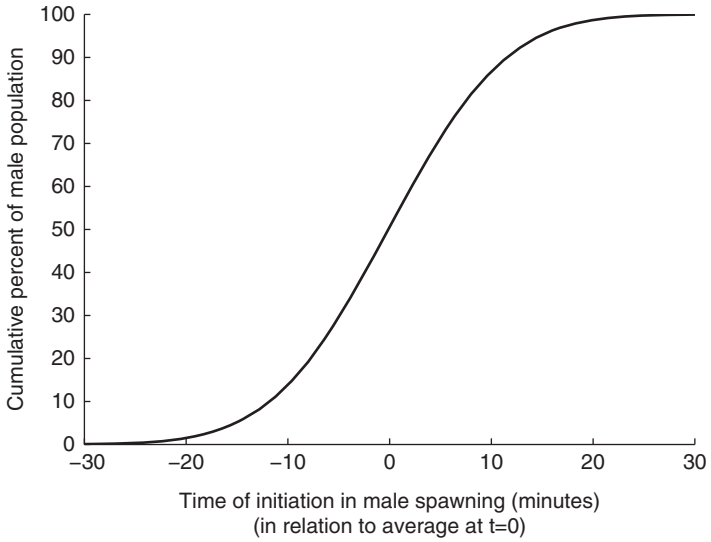

FIGURE 6.6 Cumulative distribution of the times males initiate gamete release in the model.

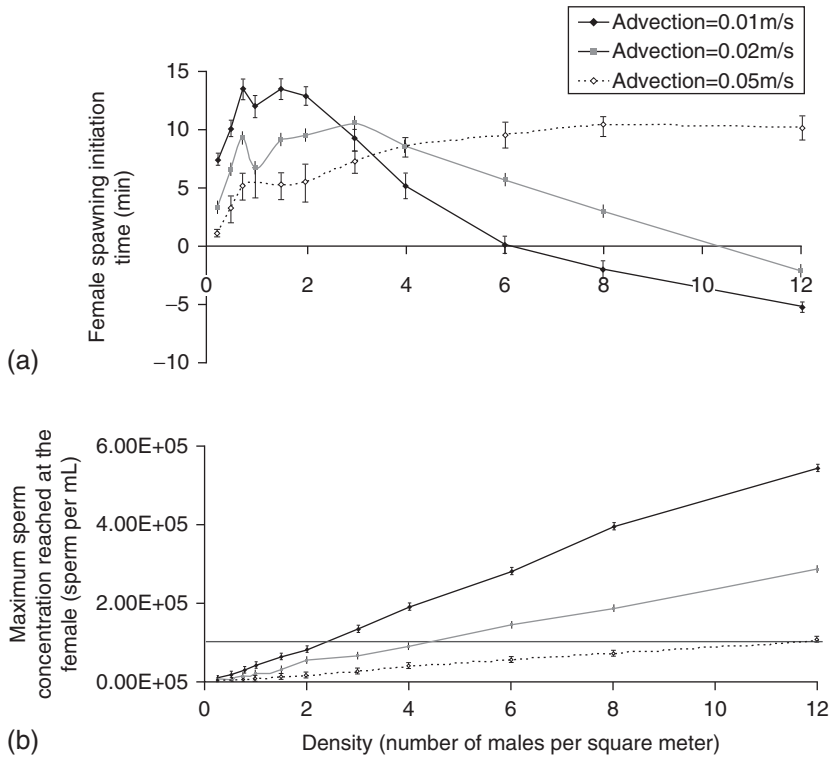

FIGURE 6.7 Model results for male spawning duration of 100 minutes. (a) Female spawning initiation is reported in relation to the average spawning in the male population, given conditions of advection and male density. (b) The maximum sperm concentration reached at the female as a function of advection and male density. The sperm threshold at which a female can get 100% of her eggs fertilized (10^5 sperm/mL) is shown by the black horizontal line.

(less that 10^5 sperm per mL), sperm concentrations are at 90% of the maximum sperm concentration reached at that trial.

When male spawning duration is long (100 minutes), female spawning initiation is the result of an interaction between male density and advection (figure 6.7a). Depending on advection, sperm can be limiting at a range of densities (below the 10^5 threshold; figure 6.7b). Inside this window of sperm-limitation, as density increases, females are predicted to wait longer for more males to spawn. This result is counter-intuitive to the notion that females should begin spawning earlier than males as density increases. This is because the amount of time males release gametes is longer than the initiation of male spawning in the population, so that the earliest males are still releasing sperm as the later males begin gamete release. However, depending on advection, eventually a density is reached when sperm becomes saturating (at the 10^5 sperm per mL threshold). Under sperm saturation, as male density increases females are predicted to initiate spawning earlier. Therefore, with increasing male density, females are predicted to delay spawning as long as sperm are limiting, and then spawn progressively sooner when sperm is saturating. The density at which this occurs is determined by advection, because it affects the rate at which sperm accumulates over the female. Higher advection shifts the transition to saturating sperm conditions (10^5 sperm/mL) to higher male densities (figure 6.7b).

We also explored how decreasing male spawning duration affects female timing in spawning (figure 6.8a, for male spawning duration of 10 minutes). Under a shorter male spawning duration, females are predicted to be more synchronous with the male population for a range of densities and advections. The decrease in female delay is caused by the relationship between male start times and duration.

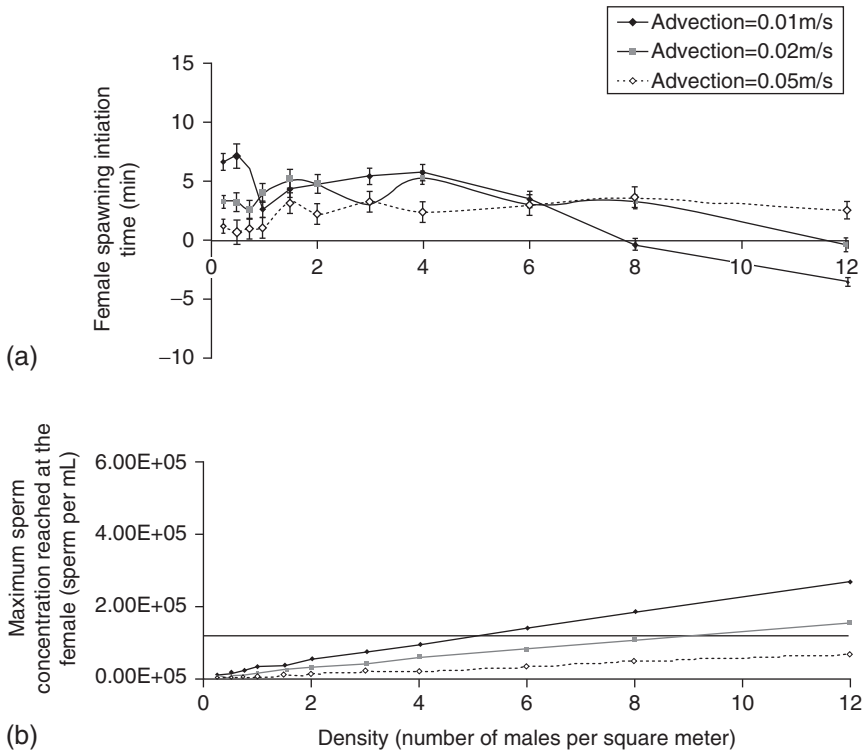

FIGURE 6.8 Model results for male spawning duration of 10 minutes. (a) Female spawning initiation is reported in relation to the average spawning in the male population, as a function of male density and advection. (b) Maximum sperm concentration reached at the female as a function of advection and male density. The sperm threshold at which a female can get 100% of her eggs fertilized (10^5 sperm/mL) is shown by the light grey horizontal line.

While start times vary among males, the female cannot wait until all males have initiated (as in the case of long male spawning duration), because by that time the early spawning males have finished spawning. This same relationship between start time and duration also results in increased variability within and among replicates. The interaction between advection and male density is more ambiguous in this case because sperm concentrations are near the threshold for a higher range of male densities (figure 6.8b).

Overall, this model suggests that there is a nonlinear effect of spawning density on male–female synchrony and this effect is mediated by water flow. In addition, the model predicts a positive relationship between male spawning duration and the delay between male and female spawning times. However in all modeled scenarios females were predicted to initiate spawning after at least some males initiated spawning.

COMPARING THE MODEL TO EMPIRICAL DATA

The model above suggests that decreasing male spawning duration results in females becoming more synchronous with the male population. We wanted to test the generality of this result for broadcast spawning animals, but data on spawning duration and sexual differences in spawning initiation are scant and difficult to collect. The only data available for testing this question is from an extensive study on reproduction of siphonous green algae in the order Bryopsidales (Clifton 1997; Clifton & Clifton 1999).

Although this volume is focused on animal taxa, several species of algae are broadcast spawners that share characteristics with animal broadcast spawners, and can provide insight into processes of fertilization, sperm limitation, and sexual selection in the sea. Clifton's data is ideal for this question, because

individuals release gametes for between 5 and 35 minutes, depending on the species. Thus green algae constitute a continuum of pluming durations. Because of the extensive survey performed by Clifton and colleagues for 20 months of nearly continuous daily monitoring, their dataset on green algae can be used as a reliable source to test the relationship between spawning duration and sexual differences in timing between males and females.

In all the dioecious green algae reported by Clifton (1997), males initiated spawning before the females. We plotted the average time difference between males and females as a function of the midpoint in spawning duration for all 15 dioecious species. There was a significant positive relationship between spawning duration and sexual differences in timing between males and females (figure 6.9; Adjusted *R*-squared = 0.49; *F*-statistic = 14.46 on 1 and 13 DF; *p*-value = 0.002).

We examined whether our model could be used to predict the differences in timing between males and females observed in Clifton's data, given a particular spawning duration in the male population. We modeled Clifton's data using an advection of 0.01 m/s, a population of 16 males in a 4-m^2 square plot, the midpoint of spawning duration observed for each algal species, and an arbitrary fixed total number of gametes for each species (10,000 gametes), such that increases in spawn duration was compensated for by a decrease in spawning rate (sperm/second). At these sperm release rates, densities, and water flows, sperm were always limiting in these simulations. The male population was modeled with 95% of males initiating spawning within a 25 minute period, which was typical of the species in Clifton's study (Clifton & Clifton 1999). Adjusting the parameter values could give better or worse fits to the empirical data, here we simply show a qualitatively similar response (figure 6.9). Most interesting would be examination of deviations from the linear predictions for each species and what factors, such as density, release rate, flow or gamete traits might explain these deviations.

CONCLUSIONS AND FUTURE DIRECTIONS

Patterns of gamete release vary among species and likely reflect differences in the selective pressures associated with successful fertilization. Experimental evidence suggests that sex differences in the timing of spawning can be influenced by the distribution and abundance of competitors and mates. At low densities selection on males will favor earlier male spawning times, because males that spawn earlier are able to spread out sperm over a greater spatial area. At low densities selection on females will

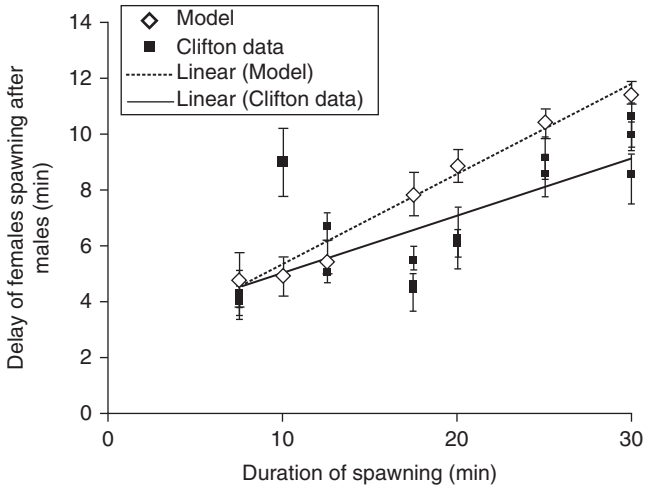

FIGURE 6.9 The relationship between spawning duration and the delay between male and female spawning. Empirical data in the green algae is presented in dark squares (Clifton and Clifton 1999); results from the model are presented in white diamonds. A significant positive relationship is observed in both the empirical and theoretical data (see text).

favor later female spawning times, because females should wait for sperm to accumulate near them as additional males join the spawning event. However, increasing mate densities will select for tighter synchrony between male and female spawning, because asynchronous males are outcompeted by more synchronous males, and sperm will accumulate above females in a shorter period of time.

Our modeling represents an early exploration into the costs and benefits of packaging gametes into long or short duration releases and how this packaging can interact with sex differences in the timing of spawning. The modeling predicts that when males have a long spawning duration, the time a female chooses to spawn is a result of a strong interaction between density and advection. Females will increasingly delay spawning with increasing density until sperm becomes saturating, at which point female delay begins to decrease. The male density at which sperm becomes saturating is larger at higher advections, because increasing advection results in lower sperm concentration at the female. As male spawning duration decreases, this interaction between density and advection becomes weaker, and female delay is shorter for a range of densities. Generally, these results predict a reduced time difference between the sexes in organisms with short spawning durations and high density populations. Comparisons of the model with data from broadcast spawning algae suggest that this approach might be fruitful, particularly when expectations fail to match predictions. More complex models and models that consider male and female strategies simultaneously (e.g., game theory) would be worth developing.

In this chapter, we documented various spawning behaviors and focused on spawning duration and its relation to sex difference in spawn time. However, this exploration is meant to stimulate additional experimental and theoretical studies. These studies need to be placed into the context of natural spawning observations. Spawning observations are still rarely reported in the literature and often these reports are not detailed enough to test the various hypotheses that might determine the costs and benefits of variation in spawning behavior or insights into how these behaviors evolve. Our hope is that this chapter motivates more quantitative measures of spawning behaviors and experiments on how various strategies influence the fertilization success of males and females.

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