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# Fish semiochemicals and the evolution of communication networks

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### Introduction

The concept that animals typically communicate in networks (involving at least one signaller and more than one receiver) derives from the active space of signals and social spacing of conspecific and heterospecific receivers (McGregor & Peake, 2000; Ch. 1). The ecological and evolutionary consequences of such networks have been explored most thoroughly for visual (e.g. Ch. 12) and acoustic signals (e.g. Otter *et al.*, 1999; Ch. 2), although it is clear that chemical signalling also can involve networks (Chs. 11 and 16). Research on aquatic communication networks has so far been limited to the context of visual and acoustic signalling (e.g. Oliveira *et al.*, 1998; Chs. 5 and 18). Semiochemicals (i.e. chemicals that transfer information within and/or between species) exert important and diverse effects on the behaviour and physiology of aquatic animals (Liley, 1982; Chivers & Smith, 1998; Kats & Dill, 1998; Sorensen & Stacey, 1999; Stacey & Sorensen, 2002; Wisenden, 2003). Studies of two key aspects of fish chemical ecology (predator-prey and reproductive interactions) have revealed great differences in the sources and nature of the semiochemicals released, their active spaces and their biological functions. These studies also provide sufficient information to assess, in fish, the existence and function of semiochemical *information networks*, which we define more fully below as a general category of network that includes not only communication networks employing specialized signals but also other networks employing unspecialized cues. Here, we briefly describe well-studied examples of predator-prey and reproductive semiochemicals to explore the applicability of current communication network theory to aquatic chemical information networks

and consider how their function and evolution might differ from those employing other sensory modalities and information-transmission media.

Research on intra- and interspecific transfer of chemical information in terrestrial species has generated a bewildering terminology related not only to the nature, actions and functions of the chemicals but also to the concept of communication (Hauser, 1996; Beauchamp, 2000; Hasson, 2000; McClintock, 2002). Although we do not presume to clarify such a complex terminological problem in this brief paper, it is imperative that we begin by clearly defining key terms, particularly as much of our subject matter appears to be distinct from that typically discussed in the context of communication networks.

#### *Definition of terminology*

Semiochemicals include *allomones* and *pheromones* that, respectively, transmit interspecific and intraspecific information. We will consider fish pheromones and allomones involved in predator-prey interactions (p. 000) separately from those involved in reproduction (p. 000). We define a pheromone as 'a substance, or mixture of substances, which is released by an individual and that evokes a specific and adaptive response in conspecifics' (Stacey & Sorensen, 2002). This definition is more inclusive than the original definition of pheromone (Karlson & Lüscher, 1959) because, for reasons explained below, it omits any requirement that pheromones be involved in *communication*. We use the terms *releaser* and *primer* not to classify pheromones but only to describe their rapid behavioural and slower physiological actions, respectively, for the simple reason that 'it is quite possible for the same pheromone to be both a releaser and a primer' (Wilson & Bossert, 1963), as is the case for sex pheromones of goldfish *Carassius auratus* (p. 000).

Central to our terminological schema is the concept (Sorensen & Stacey, 1999; Stacey & Sorensen, 2002) that evolution of chemical communication progresses through a series of three functional phases: *ancestral*, *spying* and *communication*. In the ancestral phase, individuals (*originators*) release a chemical(s) that does not influence receivers (Fig. 23.1). This primitive, prepheromonal condition progresses to spying if receivers evolve the ability to detect and respond adaptively to the originator's released chemical(s), now termed a pheromonal or allomonal *cue(s)*. In spying, originators may or may not benefit from the receiver's response but, importantly, remain in an unspecialized state with respect to production and release of pheromonal cues. Finally, spying progresses to communication if there is a mechanism for receiver responses to select for specialization in production and/or release of the detected cue(s), now termed a pheromonal or allomonal *signal(s)* and released by a *signaller*. Signals evolve through natural selection because of fitness benefits the signaller receives by manipulating the behaviour or physiology of receivers. In many cases, signal senders and receivers form a mutualism in which

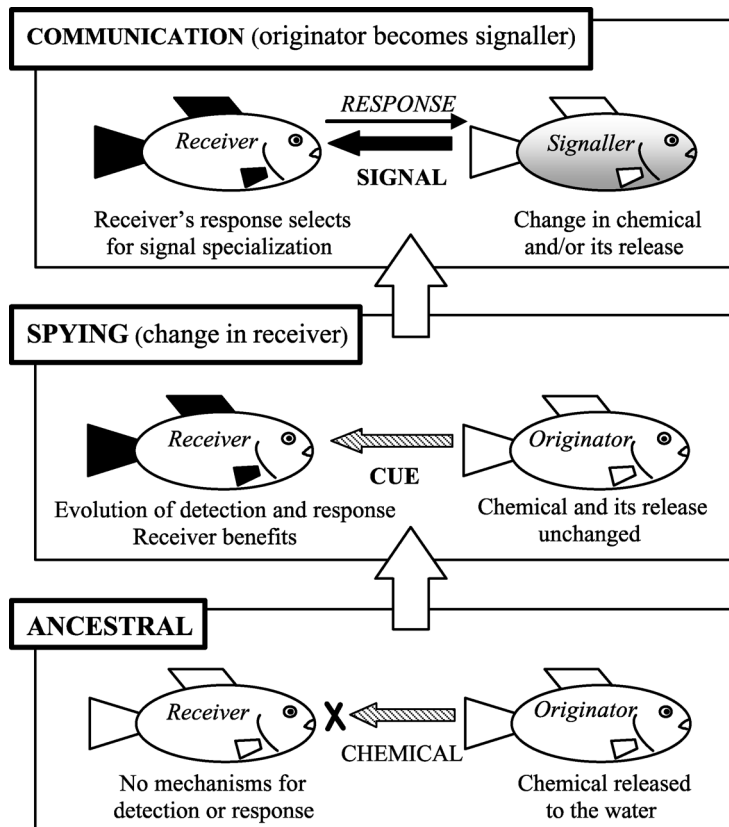


Fig. 23.1. The evolution of communication from the ancestral state, where the originator does not possess specializations for synthesis and release of semiochemicals, to spying, where receivers possess specializations for detecting semiochemicals but originators do not possess specializations, to true communication, in which both originator (now signaller) possess specializations for semiochemical exchange of information.

signals coevolve with the sensory biology of receivers. Once established, however, mutually beneficial communicative relationships could be susceptible to deceitful signal manipulations by signallers, which reduce the receiver fitness, as seen in visual, acoustic and chemical signals (e.g. Lloyd, 1965; Møller, 1989; Paxton & Tengo, 2001).

The ancestral state applies to released chemicals not currently functioning in spying or communication. We restrict the terms signal and communication to those situations in which there is clear evidence for signal specialization, such as tissue hypertrophy or discrete structures for signal production; in contrast to the situation in terrestrial insects and vertebrates, where pheromone-producing

glandular structures are common, such specializations in fish appear to be the exception rather than the rule (e.g. Laumen *et al.*, 1974; Colombo *et al.*, 1980; van den Hurk & Resink, 1992). Consequently, we regard the great majority of fish predator-prey and reproductive semiochemicals to function in spying, which is, in effect, the default condition for cases where there is no evidence for specialization in semiochemical production or release and/or where the social system apparently precludes selection for signal specialization. It is to be expected that future research may reveal some putative examples of spying to be true communication because they involve previously undetected signal specialization.

Although we believe the distinction between cues and signals is fundamental to an understanding of the function and evolution of semiochemical systems, fish olfactory systems evidently do not make this distinction and process cues and signals through similar mechanisms, which differ considerably from those processing food odours (amino acids). Therefore, in comparison with food odours, semiochemical cues and signals are detected by more sensitive and specific olfactory receptor mechanisms and generate neuronal activity that is processed in distinct arrays (glomeruli) in the olfactory bulbs, is conducted to the brain by distinct nerve bundles (olfactory tracts), and is projected to distinct brain areas (Sorensen *et al.*, 1998; Hamdani *et al.*, 2000, 2001; Brown *et al.*, 2001; Stacey & Sorensen, 2002). The distinction between chemical spying (via cues) and chemical communication (via signals) highlights a dichotomy relevant not only to our understanding of semiochemicals (the functional relationships among originators, signallers and receivers; evolutionary origins of species-specific cues and signals: Sorensen & Stacey (1999)) but also to the concept of communication networks (McGregor & Peake, 2000). In particular, first, how might networks involving communication differ from those involving spying and, second, can the concept of eavesdropping, defined as 'extracting information from signalling interactions between others' (McGregor and Peake, 2000) be applied to information networks that do not involve signalling?

#### **Transfer of chemical information**

Propagation of chemical information differs fundamentally from propagation of visual and acoustic information. In general, visual and acoustic signals are propagated with predictable speed and direction, and they generate predictable active spaces throughout which much of the temporal information contained in the signal's initial pattern can be retained. In contrast, semiochemicals are released into fluid media (air or water) in which local variation in flow typically creates turbulent odour plumes, which not only distort or destroy temporal pattern but also make the position, shape and size of the chemical's active

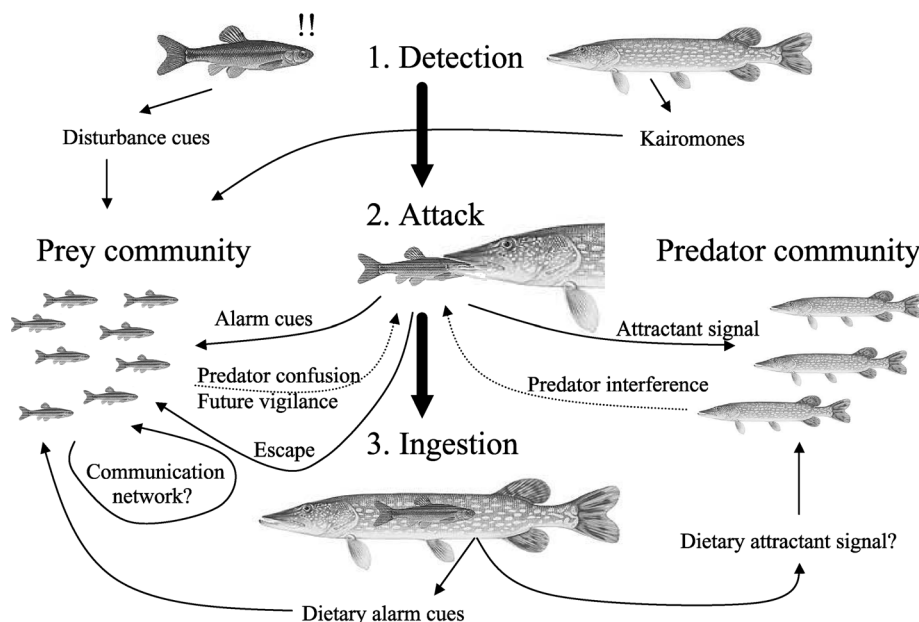
space highly unpredictable (Weissburg, 2000). Moreover, semiochemicals can persist in the environment for considerable time (e.g. Wisenden *et al.*, 1995; Sorensen *et al.*, 2000; Polkinghorne *et al.*, 2001), and thus can become disassociated from originators/signallers either when currents carry away a transiently released semiochemical or when the originator/signaller moves to a new location. Although there is considerable information on the mechanisms by which some invertebrates (e.g. crustaceans and moths) navigate in physically characterized odour plumes, this complex issue is poorly understood in fish (Vickers, 2000). Finally, it is important to realise that semiochemical function in water also can be influenced by additional solutes that affect olfactory response, such as heavy metals (Hansen *et al.*, 1999) and organics (Hubbard *et al.*, 2002).

The olfactory system is similar to other sensory systems in being functionally delimited by the sensitivity and specificity of its sensory neurons, but it differs in the nature of the information it processes. Visual and acoustic systems process linear arrays of light and sound frequencies in spectra common to many species, particularly if they are related; olfactory systems process information from odorants that cannot be arranged in a linear dimension by means of receptors that are sensitive to one or a few chemicals. These differences have two important implications for the nature and evolution of semiochemicals. First, whereas visual and acoustic signals usually encode species-typical information in frequency and temporal pattern, semiochemicals encode this information through the presence, absence or ratio of specific odorants. Second, whereas visual and acoustic signals are potentially detectable by all individuals and species sensitive to the emitted spectra, semiochemical detection will be restricted to individuals with olfactory receptors sensitive to the odorant(s). Thus, large differences in semiochemical production and detection can occur with only small changes either in chemical metabolism and release or in olfactory receptor specificity.

#### Assessment of predation risk

Temporal and spatial variation in predation risk governs much of animal behaviour. Consequently, natural selection strongly favours attendance to cues that reduce the probability of predation. Chemicals reliably inform about predation risk because they are carried well in water, persist for ecologically appropriate amounts of time, transmit information through turbid or highly structured habitat and darkness, and provide types of information not contained in visual and acoustic modalities.

To apply communication-network theory to chemical assessment of predation risk, we must first determine whether use of chemical information for the purposes of risk assessment involves spying (via cues) or communication (via signals).



**Fig. 23.2.** Semiochemicals associated with predation. Predation escalates from initial detection (top) to attack (middle) and, finally, ingestion (bottom). Chemical cues (solid arrows) released at each stage inform nearby prey (conspecific and heterospecific) and predators of the presence and extent of the interaction between predator and prey. Known and hypothesized benefits are indicated by dotted arrows.

We conclude in the discussion below that, despite a plethora of semiochemically mediated mechanisms for predator avoidance, evidence for signals is not compelling. Although these information networks may not be *communication* networks per se, there is evolutionary opportunity for communication networks and eavesdropping to evolve because receivers have evolved the ability to detect and respond to many types of semiochemical (see below).

#### *Chemicals correlated with predation*

The literature concerning chemicals linked to predation has been reviewed elsewhere (Smith, 1992; Chivers & Smith, 1998; Kats & Dill, 1998; Wisenden, 2000, 2003; Chivers & Mirza, 2001) and only a brief overview will be presented here. Several classes of chemical compound inform prey about predation risk. Generally, these cues are released passively before, during and after a predation event (Fig. 23.2). Before an attack is initiated, prey can detect and respond to three types of chemical cues: (a) odour of disturbed (startled but uninjured) prey (Chivers & Smith, 1998; Wisenden, 2003), (b) species-specific *kairomones* (a predator's natural odour) (Kats & Dill, 1998) and (c) injury-released alarm cues of prey that leak from

the gut of the predator (Chivers & Mirza, 2001). When a predator attacks and injures a prey organism, damaged prey tissues release chemical compounds that are released only in this context; consequently, these cues reliably indicate risk and elicit intense anti-predator behaviour (Chivers & Smith, 1998). These are alarm cues. Most aquatic taxa exhibit anti-predator behaviour in response to alarm cues (Chivers & Smith, 1998; Wisenden, 2003) in ways that reduce the probability of predation (Hews, 1988; Mathis & Smith, 1993; Wisenden *et al.*, 1999; Gazdewich & Chivers, 2002). Ingested prey release chemical information in the form of alarm cues, or their metabolites, from the gut of their predators (Chivers & Mirza, 2001). The ecological reality is undoubtedly more complex than the interactions depicted in Fig. 23.2. Additional interactions arise from variation in diet overlap among predators, threat from each predator species over time and space, and interacting ontogenies of prey and predator species.

So far, only one of these semiochemicals qualifies as a specialized signal (see p. 000). The vast majority of chemical information used by aquatic prey to assess predation risk appears to be opportunistic use of chemical information mediated by unspecialized chemical cues. This information is of great fitness benefit to receivers, but receiver response generally has not been shown to accrue benefit to the originator/signaller. This system might be described most parsimoniously as an information network, where a suite of predator species coexists with a suite of prey species.

#### *Ostariophysan alarm substance cells*

For passively released chemical cues to qualify as signals, specializations for their synthesis and/or release must occur that plausibly have been selected for by benefits accruing to the originator/signaller. This condition appears to be met in fishes of the superorder Ostariophysi (minnows, tetras, catfishes, suckers and sundry others). This large group of vertebrates (> 5500 species) makes up approximately 27% of the global ichthyofauna and 64% of all freshwater fish species (Nelson, 1994). In addition to successful occupation of a diverse array of habitats, they are often the numerically dominant vertebrates in aquatic ecosystems.

Ostariophysans possess specialized epidermal cells that contain a potent alarm chemical(s), termed *schreckstoff* or alarm substance (von Frisch, 1941; Pfeiffer, 1977; Smith, 1992); this appears to activate components of the olfactory system that also are activated by sex pheromones (Hamdani *et al.*, 2000). It is not known how much skin area is typically damaged during a predatory attack, but homogenates of 1 cm<sup>2</sup> skin can create active spaces of 10 000 litres (zebrafish *Danio rerio*; Gandolfi *et al.*, 1968) to 58 000 litres (fathead minnow *Pimephales promelas*; Lawrence & Smith, 1989), equivalent to spheres 2.6–4.8 m in diameter. The active ingredient in ostariophysan alarm cells is likely, at least in part, to be hypoxanthine 3*N*-oxide,

a compound first isolated from European minnows *Phoxinus phoxinus* (Argentini, 1976; after Smith, 1999). Subsequent work demonstrated that hypoxanthine 3N-oxide elicits anti-predator behaviour from a characin (Pfeiffer *et al.*, 1985) and from fathead minnows at concentrations as low as 0.4 nmol/l (Brown *et al.*, 2001). However, efforts to detect hypoxanthine 3N-oxide in fathead minnow skin with high performance liquid chromatography have not been successful (Smith, 1999) and fractionation of skin extract indicates the biologically active component is found with the polypeptides with molecular weights greater than 1100 (Kasumyan & Ponomarev, 1987) rather than as a small molecule such as hypoxanthine 3N-oxide. It is possible that hypoxanthine 3N-oxide is associated with protein while within skin cells and remains associated with protein once released. Heated-treated skin extract of fathead minnows loses 70% of its protein and its ability to elicit alarm (N. L. Korpi, L. D. Louisiana, J. J. Provost & B. D. Wisenden, unpublished data). A protein-hypoxanthine association would be consistent with cross-species alarm reactions that decline with phylogenetic distance (Schutz, 1956). Whatever the active ingredient(s) of alarm cue might be, their biological potency (Lawrence & Smith, 1989; Brown *et al.*, 2001) suggests selection for olfactory sensitivity similar to that seen with sex pheromones (Stacey & Sorensen, 2002).

Is ostariophysan alarm substance a case of communication? Although the epidermal cells appear to be structures specialized for information transfer of alarm, selection for signal specialization via benefits to the originator (i.e. the individual that released the substance) is not immediately apparent. There has been much speculation over the historical and current selection benefits to individuals that invest in these cells (Smith, 1992, 1997; Williams, 1992; Magurran *et al.*, 1996; Henderson *et al.*, 1997). Smith (1992) summarized 16 hypotheses by which signallers may benefit from alarm signalling, only one of which, attraction of secondary predators, has been tested for chemical alarm cues. Laboratory and field experiments have demonstrated that predators are attracted to minnow skin extract (Mathis *et al.*, 1995; Wisenden & Thiel, 2002) and that interruption of a predation event by the arrival of a second predator allows prey an opportunity to escape (Chivers *et al.*, 1996), a benefit that elevates passively released cues to signal status. However, it is not an alarm signal, but an attractant signal. The signaller benefits from the responses of secondary predators, not from responses of conspecific and heterospecific members of the prey community.

Is this a communication network? Are members of the prey community eavesdroppers on the predator-attractant signal? Assessment of predation risk via chemical cues does not depend on receiver (secondary predators) response (i.e. not social eavesdropping but interceptive eavesdropping (defined in Ch. 2) of this attractant signal provides highly salient temporal and contextual information about predation risk. Therefore, from the perspective of the general non-ostariophysan

prey community, detection of injury-released chemical compounds may be considered as (interceptive) spying; however for the ostariophysan fishes, spying on the predator-attractant signal might best be considered as a case of interceptive eavesdropping (Ch. 2).

*Evolutionary opportunities for communication networking*

There is field evidence that prey fishes frequently survive predatory attacks (Smith & Lemly, 1986). If an originator/signaller is injured by a predator but survives the attack, it may benefit from group behavioural responses of the prey community (Smith, 1992; Fig. 23.2). A surviving alarm signaller can accrue fitness benefits by inducing in the nearby prey community increased shoal cohesion and dashing or skittering behaviour to confuse predators and reduce attack efficiency. Minnows associate alarm cues with correlates of predation such as predator appearance and odour (reviewed by Chivers & Smith, 1998). Alarm cues enable conspecifics and heterospecifics to acquire predator recognition after a single simultaneous or non-simultaneous encounter with a novel indicator of risk (Suboski, 1990; Suboski *et al.*, 1990; Chivers & Smith, 1994; Hall & Suboski, 1995; Korpi & Wisenden, 2001). Therefore, a third benefit to the signaller would be providing shoalmates an opportunity to learn predator identity, as a shoalmate trained in this way may detect that predator in the future and alert the signaller. If a shoal contains individuals related to the signaller, then a fourth benefit might accrue to the signaller's inclusive fitness through kin selection.

Several lines of evidence suggest potential for eavesdropping on signaller-group communication in the ostariophysan system. Alarm responses by minnow shoals induce anti-predator behaviour in conspecifics in adjacent aquaria with visual but not chemical contact with the alarmed shoal (Verheijen, 1956; Magurran, 1989; Suboski *et al.*, 1990; Brown *et al.*, 1999). Predator-naïve eavesdroppers can acquire recognition of novel predators by associating the behaviour of alarmed shoalmates with novel correlates of predation (Suboski *et al.*, 1990; Mathis *et al.*, 1996).

Although evolutionary ecologists have focused on cells producing alarm substances in ostariophysans, these fishes are not unique in possessing specialized epidermal cells (Smith, 1992). The epidermal layer of freshwater perch, wall-eye and darters (superorder Acanthopterygii, order Perciformes, family Percidae: Smith, 1979, 1982; Wisenden, 2003), Australian bullies (order Perciformes, family Eleotridae: E. A. Kristensen, personal communication) and poeciliids (superorder Acanthopterygii, Order Cyprinodontiformes, family Poeciliidae: Bryant, 1987) all possess epidermal club cells with similar histological properties. The tropical marine and freshwater fishes in the Gobiidae (order Perciformes, 1875 species) possess epidermal vacuolate cells but have an inconsistent behavioural response to skin

extract (Smith, 1992). This leaves open the possibility that analogous communication networks for assessment of predation risk occur among other fish taxa.

In summary, it is parsimonious, based on current knowledge of chemical alarm cues, to conclude that for aquatic taxa, including most fishes, chemically mediated risk assessment does not constitute a true communication network because it is not based on specialized signals. However, the ostariophysan alarm semiochemical system appears to be a good candidate for an incipient communication system (see p. 000). Future research may reveal signaller–group communication of alarm and potentially uncover communication networks and eavesdropping.

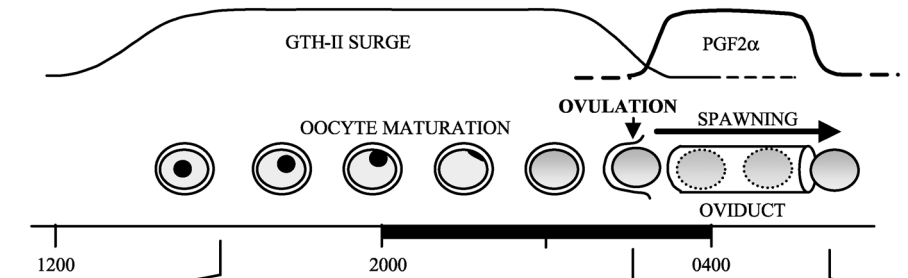
### Sex pheromones in information networks

In addition to alarm responses discussed in the previous section, pheromonal cues and signals of teleost fish influence many diverse non-reproductive (migration, parent–young interactions, schooling and related social behaviours: Liley, 1982) and reproductive (Stacey *et al.*, 1986; Stacey & Sorensen, 2002) phenomena. Best understood are those cases (the great majority being reproductive) in which chemical identification has allowed study of pheromone production, detection and biological effects under controlled and repeatable conditions. Since Colombo *et al.* (1980) first proposed that a conjugated steroid (eti-cholanolone glucuronide) is a male sex pheromone that attracts the female black goby *Gobius niger* to his nest for spawning, many studies have reported putative pheromonal roles for steroid and prostaglandin hormones, and their precursors and metabolites (hereafter termed *hormonal pheromones*) in a variety of fish (reviewed by Sorensen & Stacey, 1999; Stacey & Sorensen, 2002). Indeed, we expect the use of hormonal pheromones might be universal among fish, given that information-rich hormones and hormonal metabolites are necessarily released into the same water medium bathing the olfactory systems of conspecifics. Here, we briefly discuss two species in which identification of distinctly different reproductive pheromones has led to an understanding of pheromone function germane to concepts of chemically mediated information networks.

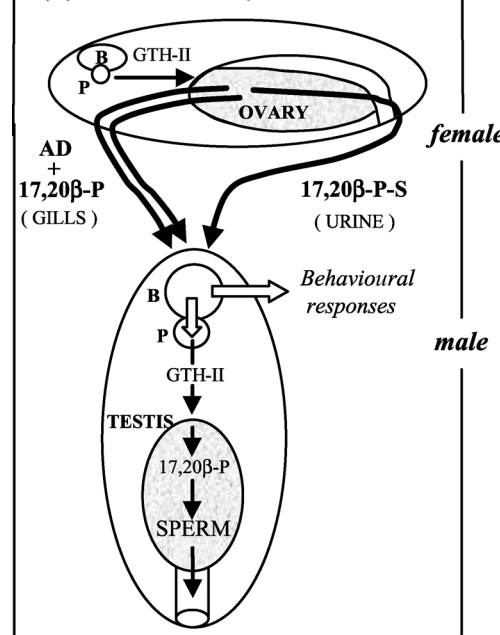
#### *Goldfish*

The hormonal pheromones of goldfish are currently the best understood of any fish and have recently been reviewed in detail (Sorensen & Stacey, 1999; Stacey & Sorensen, 2002); therefore, we provide a brief summary before considering aspects that appear directly related to concepts of semiochemical information networks. Goldfish live in mixed-sex, apparently unstructured, groups, undergoing gonadal growth during the winter and spawning a number of times in spring and summer. At ovulation, which occurs near dawn, groups of males vigorously

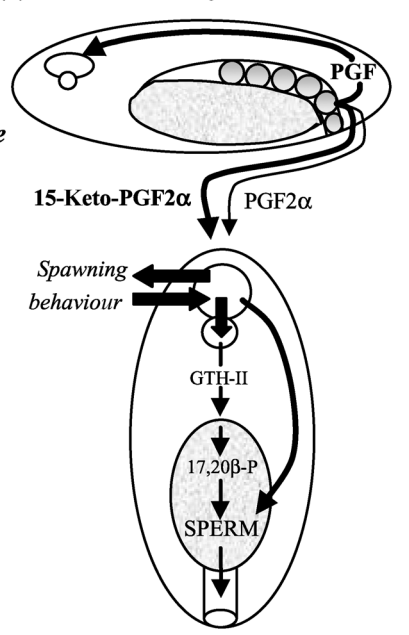
**(a) Female Periovolutary Events**



**(b) Preovulatory Pheromone**



**(c) Postovulatory Pheromone**



**Fig. 23.3.** Nature and actions of goldfish hormonal pheromones released by periovolutary females (see Stacey & Sorensen (2002) for additional details and original sources). (a) Female periovolutary events. An afternoon surge of pituitary (P) gonadotrophin II (GTH-II) release induces follicular synthesis of  $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one ( $17,20\beta$ -P), which induces final maturation (completion of arrested meiosis) in mature oocytes. When ovulation occurs approximately 12 hours later, oocytes in the oviduct stimulate synthesis of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ), which remains at high concentrations in the blood until ovulated oocytes are shed. (b) Preovulatory pheromone. During the GTH-II surge, females release a changing mixture of three steroids:  $17,20\beta$ -P and androstenedione (AD), which are released together across the gills, and a sulphated  $17,20\beta$ -P metabolite ( $17,20\beta$ -P-S), which is released in urine pulses. Peak release of AD (which inhibits endocrine response to  $17,20\beta$ -P) occurs early in the GTH-II surge, followed by peaks of  $17,20\beta$ -P and  $17,20\beta$ -P-S release. The preovulatory steroid acts on specific and sensitive (picomolar detection threshold) olfactory receptors, both inducing male behavioural responses and, by the time of ovulation, increasing the quantity and quality of sperm

compete for spawning access as females repeatedly enter aquatic vegetation to oviposit adhesive, undefended eggs over a period of several hours. In such a promiscuous mating system, where male reproductive success likely depends only on the number of eggs fertilized, we believe sperm competition has been a major selective force in the evolution of male reproductive tactics.

The cascade of events leading to spawning begins when exogenous factors (increased water temperature and aquatic vegetation) trigger an afternoon surge release of pituitary gonadotrophin II (GTH-II), which stimulates follicular synthesis of the oocyte maturation-inducing steroid  $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one ( $17,20\beta$ -P) (Fig. 23.3a). Ovulation occurs approximately 12 hours later; at which point females become sexually active for the several hours that eggs in the oviduct stimulate synthesis of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ), a behavioural hormone (Fig. 23.3c). During the approximately 15 hours between the onset of the GTH-II surge and completion of spawning, females sequentially release a preovulatory steroid pheromone (Fig. 23.3b) and a postovulatory prostaglandin pheromone (Fig. 23.3c), which dramatically affect male physiology and behaviour.

The preovulatory steroid pheromone (Fig. 23.3b) is a dynamic mixture in which the primary components appear to be  $17,20\beta$ -P, its sulphated metabolite ( $17,20\beta$ -P-S) and androstenedione (a testosterone precursor). Although the nature and actions of the preovulatory pheromone are complex (Stacey & Sorensen, 2002), it induces in males both releaser effects on socio-sexual behaviours (e.g. Poling *et al.*, 2001) and a dramatic primer effect: a rapid increase in blood GTH-II that increases both the quantity and quality of releasable stores of milt (sperm and seminal fluids) in the sperm ducts prior to ovulation and spawning (e.g. Zheng *et al.*, 1997).

At ovulation, females terminate release of the preovulatory steroid pheromone and begin to release the postovulatory prostaglandin pheromone ( $PGF_{2\alpha}$  and its more potent metabolite 15-keto- $PGF_{2\alpha}$ ) (Fig. 23.3c). The prostaglandin pheromone not only triggers male courtship and attracts the male to the ovulated female (anosmic males do not spawn) but also activates non-endocrine and endocrine

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**Fig. 23.3 (cont.)** stores in the ducts (inducing GTH-II release, which stimulates testicular  $17,20\beta$ -P synthesis). (c) Postovulatory pheromone. Entry of ovulated oocytes to the oviduct stimulates synthesis of  $PGF_{2\alpha}$ , which acts in the brain (B) to stimulate female sexual behaviours.  $PGF_{2\alpha}$  and its more potent metabolite 15-keto- $PGF_{2\alpha}$  are released in urinary pulses and act on olfactory receptors to trigger male sexual behaviours. Sexual interactions then stimulate movement of sperm to the ducts by two mechanisms: an endocrine mechanism distinct from that mediating testicular response to the preovulatory pheromone; and a rapid and apparently non-endocrine mechanism that begins to increase sperm stores within 15 minutes.

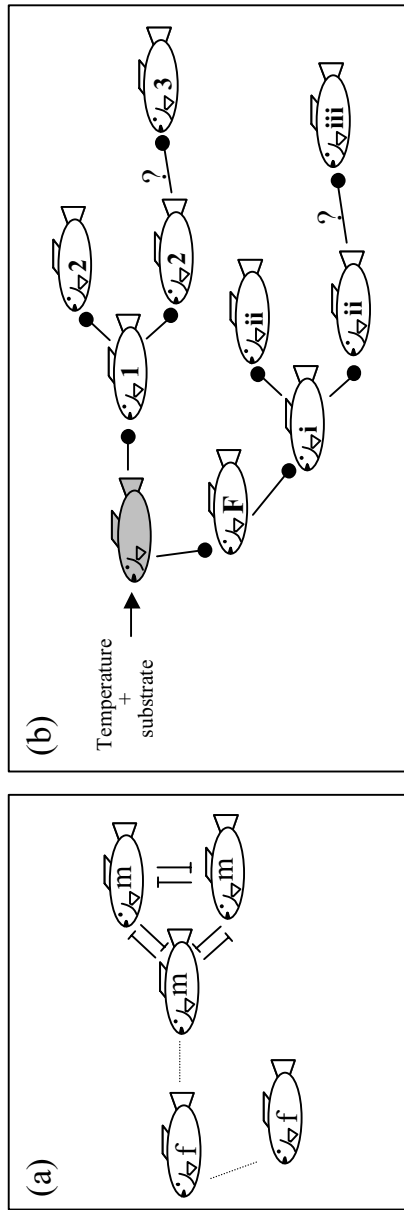
mechanisms (different from those mediating responses to the preovulatory pheromone) that further increase the volume of releasable milt.

In summary, male goldfish first increase their potential fertility through endocrine responses to reliable chemical indicators of imminent ovulation (17,20 $\beta$ -P and 17,20 $\beta$ -P-S) and then use reliable indicators that ovulation has occurred (PGF2 $\alpha$  and 15-keto-PGF2 $\alpha$ ) to locate the female and maintain sperm stores. For a number of reasons (Stacey & Sorensen, 2002), most notably a lack of evidence for specialized pheromone production and release, we regard these components of the goldfish hormonal pheromone system as an example of male spying on female chemical cues. Although it is difficult to exclude the possibility that domestication has influenced the goldfish pheromone system, it appears remarkably similar to those of the closely related Crucian carp *Carassius carassius* and common carp *Cyprinus carpio* (Irvine & Sorensen, 1993; Stacey *et al.*, 1994; Bjerselius *et al.*, 1995). Furthermore, it is likely that other cyprinids (Family Cyprinidae; > 2000 species) possess similar pheromone systems given that olfactory detection of 17,20 $\beta$ -P-like steroids and prostaglandins is widespread among this taxon (Stacey & Sorensen, 2002).

The effects of goldfish pheromones described above have been studied in the context of dyadic interactions between female originators of hormonal pheromone cues and their male receivers (Fig. 23.3b,c). However, given the proximity of individuals in aggregations, and the size of pheromonal active spaces estimated from release rates and olfactory detection threshold (Sorensen *et al.*, 2000), it is obvious that these ovulatory cues normally operate in an information network, where a female's preovulatory steroids can potentially be detected by many males and her postovulatory prostaglandins are the proximate trigger promoting sperm competition at spawning. Moreover, the network activated by the preovulatory pheromone evidently includes not only the ovulatory female and her potential spawning partners but also additional females and males not directly exposed to her preovulatory cues (Fig 23.4.).

The evidence for female interactions is based on the finding that low concentrations of water-borne 17,20 $\beta$ -P induced ovulation in goldfish (Sorensen & Stacey, 1987), suggesting a mechanism for the ovulatory synchrony observed in the field and laboratory. The female benefit(s) of ovulatory synchrony is not known but may involve predator swamping, amplification of preovulatory cues that stimulate male fertility, or (perhaps counter-intuitively) reduction of male to female ratios at spawning (high ratios can result both in 'forced' egg release away from suitable spawning substrate and skin damage through abrasion by the male's breeding tubercles or 'pearl organs').

Interactions among males appear more complex because they both decrease (Fig. 23.4.a) and increase sperm stores in response to unidentified cues from other males (Stacey *et al.*, 2001; Fraser & Stacey, 2002). For example, males isolated from



**Fig. 23.4.** Network activation by pheromones. (a) In the presence of mature females that are not ovulatory (f), mature males remain in a 'basal' endocrine state (m) and release unknown cues that suppress sperm stores. (b) If exogenous stimuli (increased water temperature and aquatic vegetation) induce an ovulatory gonadotrophin II surge in one male (1) that increase his sperm store; he than the preovulatory pheromone can directly stimulate endocrine changes in additional males (2). In addition, the preovulatory pheromone can induce releases unknown cues inducing similar changes in additional males (2). In addition, the preovulatory pheromone can induce ovulation in additional females (F), whose preovulatory pheromone release will activate additional networks of directly (i) and indirectly (ii) stimulated males. It is not known if males (2 and ii) that receive indirect information that ovulation is imminent in turn influence additional males (3 and iii).

a male group dramatically increase sperm stores within 24 hours, indicating they normally suppress their potential for milt production in response to an inhibitory male cue(s). However, if sperm stores of one of a group of males are increased (either by gonadotrophin injection or exposure to 17,20 $\beta$ -P), untreated males in the group also increase their stores. It, therefore, appears that, in the absence of cues from preovulatory or ovulated females, a mature male goldfish is both originator and receiver of unknown cues that suppress sperm stores in other males by maintaining basal GTH-II and steroids (Fig. 23.4.a). This stable, negative-feedback situation is rapidly and transiently perturbed, however, when exogenous stimuli trigger a preovulatory GTH-II surge in females, resulting in release of the preovulatory steroid pheromone. Males and non-ovulatory females encountering this stimulatory cue in turn increase their GTH-II, amplifying and disseminating the original cue(s) and promoting synchronous final maturation (ovulation and increased sperm stores) of individuals within the network (Fig. 23.4.b).

Numerous unresolved questions make it difficult to compare the complex reproductive interactions of goldfish with the classical visual and acoustic communication networks that have been studied in terrestrial species. Perhaps the key issue is whether the pheromonal interactions known among goldfish involve only responses of receivers to unspecialized cues, or whether some are mediated by specialized signals. There is no evidence that female preovulatory and postovulatory pheromones are specialized signals to males or other females (Sorensen & Stacey, 1999; Stacey & Sorensen, 2002). Nor is it obvious how the male's adaptive endocrine–testicular response to female preovulatory cues (Fig. 23.3b) would also be shaped by selection to include the release of a specialized signal that evidently increases the fertility of his competitors. Indeed, it seems more probable that the indirect responses of males (2 and ii in Fig. 23.4.b) to female preovulatory cues are mediated by spying on unspecialized cues released as by-products of the endocrine responses of males (1 and i in Fig. 23.4.b) directly stimulated by a preovulatory female. In a species where males are territorial and where females mate with several males on their territories, the interactions depicted in Fig. 23.4.b might be expected to have arisen from female tactics to promote sperm competition. However, given that the female goldfish cannot control the number of males competing for fertilization attempts, that virtually all her eggs can be fertilized by a single male (Zheng *et al.*, 1997) and that, as noted above, additional males may disrupt spawning activity, we feel it most probable that the interactions depicted in Fig. 23.4.b result solely from male competition.

We hope that our proposal that the hormonal pheromones of goldfish function in spying interactions will stimulate discussion of how this and similar systems can be integrated into current theoretical concepts of information networks based

on true communicative interactions. To open such a discussion, can we speak of eavesdropping in spying networks (e.g. males 2 and ii in Fig 23.4.), given that eavesdropping appears to be restricted to interactions mediated by specialized signals (McGregor & Peake, 2000; Ch. 2) or do we require new terms and/or new definitions?

*Sea lamprey *Petromyzon marinus**

Anadromous sea lamprey *Petromyzon marinus* spend most of their life as stream-dwelling, filter-feeding ammocoete larvae before undergoing a dramatic metamorphosis, migrating to the ocean or large lakes and feeding parasitically on large fish, whose unpredictable movements can carry the lamprey far from their natal streams. After approximately a year, the parasites cease feeding, begin to mature sexually and search for a spawning stream, guided by a potent pheromone that serves as a reliable indicator of suitable larval habitat. Since gaining access to the American Great Lakes from the Atlantic Ocean about a century ago, the sea lamprey has seriously depleted many of these lakes' fisheries. Based on preliminary evidence (Teeter, 1980) that larvae release a pheromone attracting migrating adults and that spawning adults employ sex pheromones, sea lamprey pheromones have been extensively studied in the hope of identifying semiochemicals for use in biological control, as has successfully been achieved for many insects (Chapman, 2000).

Larval pheromone attracting migratory adults

Both field and laboratory studies provide compelling evidence that migratory adult lamprey do not return preferentially to natal streams but instead locate suitable spawning habitat by responding to a pheromone released by stream-dwelling larvae. Historical capture records show that estimated numbers of migrating adults fall by up to 50% following application of larvicides to remove larval populations (reviewed by Sorensen & Vrieze, 2003). Evidence that such reductions in migrant numbers result from removal of larval odour comes from studies of captive migrants in large two-choice mazes (Vrieze & Sorensen, 2001). Water from streams without larvae is much less attractive to adults than is water from larva-bearing streams, but water from streams without larvae becomes attractive following addition of low concentrations of larval odour. The potency of larval odour is such that a single larva (weighing only several grams) creates an active space of 400–4000 l/h, sufficient to account for the attractive properties of streams with larvae. These studies also reveal that spawning-stream selection is based on more than larval odour alone: migratory adults prefer stream water (even

without larval odour) to lake water, suggesting the presence of unknown stream odorants that act synergistically with larval odour (Vrieze & Sorensen, 2001).

The larval pheromone attracting adult migrants has been fully characterized and shown to be a mixture of chemicals (Vrieze & Sorensen, 2001; P. W. Sorensen, personal communication); two of the primary components are the novel bile acids, allocholic acid (ACA;  $3\alpha,7\alpha,12\alpha$ -trihydroxy- $5\alpha$ -cholan-24-oic acid) and petromyzonol sulphate (PS;  $3\alpha,12\alpha,24$ -trihydroxy- $5\alpha$ -cholan-24-sulphate). PS may be a unique lamprey product and is synthesized by the liver of larvae but not by the parasitic or adult phases (Polkinghorne *et al.*, 2001). Because larvae undergo gall bladder and bile duct atrophy at metamorphosis and also cease synthesis of PS and ACA (Polkinghorne *et al.*, 2001), these compounds should be specific indicators of streams containing favourable spawning and nursery habitat. PS and ACA, which are released primarily in larval faeces (Polkinghorne *et al.*, 2001), are detected by the olfactory organ of migratory adults (Li & Sorensen, 1997) not only with great specificity, but also with a sensitivity (1 pmol/l olfactory detection threshold) that would account for behavioural responsiveness at the low concentrations estimated to occur in spawning streams (Polkinghorne *et al.*, 2001). Furthermore, these bile acids attract migratory adults (but not parasites) in maze tests (Bjerselius *et al.*, 2000; Vrieze & Sorensen, 2001).

Taken together, the results indicate that a suite of conspecific cues regulate stream selection and upstream migration of maturing adult lamprey, and that response to larval odour is adaptive in so far as it increases the likelihood of locating habitat suitable for larval growth. Moreover, because there is no evidence at this time that larval production and release of PS and ACA are specialized for functions other than digestion (Polkinghorne *et al.*, 2001), and no evident mechanism whereby adult response could select for specialized signalling functions for these compounds, we regard these components of the migratory pheromone as cues involved in chemical spying. Unlike the transient pheromonal steroid and prostaglandin cues of goldfish, which are released only at specific stages of reproduction, however, the bile acid cues of lamprey appear to be released not only during the period of peak adult migration in May but throughout the extended period (April–August) of larval feeding (Sutton & Bowen, 1994; Polkinghorne *et al.*, 2001). In addition, whereas the transient pheromonal cues of female goldfish are estimated to generate only small active spaces (Sorensen *et al.*, 2000), PS and ACA released by larval lamprey are estimated to create very large active spaces sufficient to serve effectively as an upstream attractant given that larval populations can contain hundreds of thousands of individuals (Polkinghorne *et al.*, 2001). Perhaps the greatest departure from the goldfish situation, however, is that, whereas goldfish pheromonal cues promote interactions of originators and receivers within a small social unit, the lamprey larval pheromone functions in

a vast network of dispersed originators and receivers that do not interact behaviourally.

#### Sex pheromones

During upstream migration, adult male and female lamprey undergo final maturation (spermiation and ovulation), lose behavioural responsiveness to the larval pheromone and develop behavioural responsiveness to the odour of mature conspecifics of the opposite sex (Bjerselius *et al.*, 2000; Li *et al.*, 2002). Although the described behavioural responses of mature adults (positive rheotaxis, increased locomotory behaviours) are rather non-specific, they are appropriate to mediate upstream movement to spawning grounds and facilitate male–female interactions, although this has not been demonstrated experimentally. However, the traditional use of mature males to trap females (Fontaine, 1938; discussed in Teeter, 1980) supports the existence of a potent male attractant, which is the only lamprey sex pheromone to be studied intensively. This pheromone, estimated to have a large active space ( $> 10^6$  l/h per adult male (Li *et al.*, 2002)), is proposed to function in attracting females to mature males, which are reported to precede females to the spawning grounds.

Major components of the pheromone released by spermiated male lamprey are proposed to be 3-keto-petromyzonol-sulphate (3-keto-PS;  $7\alpha,12\alpha,24$ -trihydroxy-3-one-5 $\alpha$ -cholan-24-sulphate) and 3-keto-allocholic acid (3-keto-ACA;  $7\alpha,12\alpha$ -dihydroxy-5 $\alpha$ -cholan-3-one-24-oic acid) (Li *et al.*, 2002; Yun *et al.*, 2003). Although both these compounds are detected by the lamprey olfactory system, only 3-keto-PS has been investigated for pheromonal activity. As with the odour of spermiated males, 3-keto-PS when added to a two-choice maze both attracts ovulated females (but not preovulatory females or males) and stimulates their searching behaviours (Li *et al.*, 2002). Moreover, whereas non-spermiated males (whose odour does not attract ovulated females in the maze) do not release appreciable quantities of 3-keto-PS, spermiated males release large quantities of 3-keto-PS (approximately 500 g/h) (Li *et al.*, 2002; Yun *et al.*, 2002).

As with the bile acid pheromone of larval lamprey (Polkinghorne *et al.*, 2001), 3-keto-PS has been found in the liver of spermiated males (Li *et al.*, 2002). However, unlike the larval pheromone, which is released primarily in faeces, the pheromone from spermiated males appears to be released by the gills, which in mature males (but not females) develop glandular cells (Pickering, 1977) that have been suggested to be specialized for pheromone release (Li *et al.*, 2002). This possibility deserves consideration, given the scant evidence for such structural specializations in fish pheromone systems.

The current information on male lamprey pheromone suggests its synthesis occurs through a subtle shift in bile acid metabolism that results in the larval

pattern of PS and ACA production changing to 3-keto-PS and 3-keto-ACA in spermiating males (presence of the 3-keto acids in livers of ovulating females appears not to have been examined). Furthermore, because fully mature adults are exposed to larval and adult bile acids in spawning streams, it is expected that the lamprey olfactory system has been selected to discriminate larval (3-hydroxy acid) and adult (3-keto acid) odours, although this remains to be examined.

The identified sex pheromones of lamprey and goldfish are similar in that they operate within a complex network of originators/signallers and receivers, although they differ fundamentally both in the interactions between genders and in the ancestral (prepheromonal) functions of the cues and signals. Moreover, the possibility of signal specialization in production and release of male lamprey pheromone suggests a true communicatory interaction, which is unlikely in goldfish.

### Synthesis

Current theory about the function of animal communication networks (e.g. McGregor & Peake, 2000) has been heavily influenced by studies of acoustic and visual systems, where it seems clear that true communication between specialized signallers and receivers has arisen through the bilateral benefits resulting from their reciprocal interactions. Although studies of fish semiochemicals also provide evidence of specializations indicative of communication, the specific functions of such specialized semiochemicals within networks are not well understood.

In sea lamprey, for example, both the large active space of the proposed male sex pheromone 3-keto-PS and apparent male-specific gill structure facilitating its release (Li *et al.*, 2002) suggest specializations for increased amplitude of a specialized tonic signal. The proposed function of this male lamprey signal appears analogous to the aggregate signal produced by chorusing male anurans (Ch. 13), in so far as the combined odour of many males induces the upstream movement of many females. However, it remains to be determined if attracted female lamprey also use the male pheromone in mate choice and if this might have been the pheromone's original function.

Also, in the black goby, non-spermatogenic portions of the testes appear specialized for synthesis of a steroid pheromone, etiocholanolone glucuronide, originally proposed simply to attract ovulated females to the male's nest (Colombo *et al.*, 1980). In the round goby *Neogobius melanostomus*, however, both males and females respond behaviourally to etiocholanolone glucuronide (Murphy *et al.*, 2001), suggesting that the pheromone functions in a more complex network involving both intra- and intersexual communication.

Given that semiochemical communication appears to have evolved in sea lamprey and gobies, and perhaps in some other fish such as blennies (Laumen *et al.*,

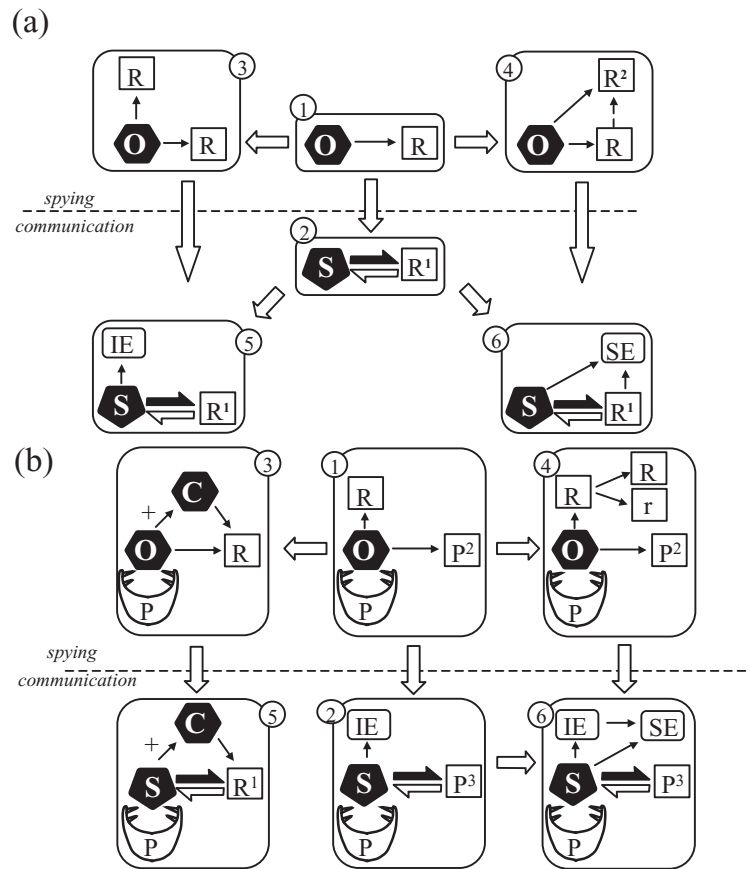
1974; Gonçalves *et al.*, 2002) and African catfish *Clarias gariepinus* (van den Hurk & Resink, 1992), these species may communicate in semiochemical networks analogous to those seen in terrestrial systems involving acoustic and visual signals. However, other fish semiochemicals, such as the alarm cues of ostariophysans and the sex pheromones of goldfish, appear to function not in communication but rather in spying, where specialization for information transfer evidently is restricted to receivers. Nonetheless, these semiochemical cues also operate in complex information networks in which semiochemicals can influence several conspecifics both directly (through exposure) and indirectly (through changes induced in exposed individuals) (e.g. Figs. 23.2 and 23.4.b)

Because such fish semiochemical networks based on unspecialized cues have the potential to give rise to true communication networks, they should not only extend the scope of current network theory but also raise important issues relevant to the evolutionary processes by which such communicatory networks evolve. To cite just one example, when discussion of information networks is restricted to those that involve communication, it might seem reasonable to assume that eavesdropping arises only after communicative interaction has been established. However, the ability of male goldfish to derive information indirectly about female cues by spying on the responses of exposed males (e.g. Figs. 23.2 and 23.4.b) demonstrates that a process analogous (and possibly homologous) to eavesdropping can precede the origin of communication.

To promote discussion of the functional and evolutionary relationships among spying, eavesdropping and communication, we propose two hypothetical schemes. One is based on the intraspecific interactions induced by the goldfish preovulatory steroid pheromone (Fig. 23.5a); the second involves both intra- and interspecific predator-prey interactions in ostariophysan fishes (Fig. 23.5b), and both are derived from our general model for the evolution of communication (Fig. 23.1).

In goldfish, spying by male receivers (R) on an unspecialized steroid cue released by female originators (O; Fig. 23.5a1) *could* lead to communication (Fig. 23.5a2) if male response to heritable variation in cue production leads to differential female fitness. If this occurs, females would then be signallers (S) releasing a specialized pheromonal signal and the male's role would change (R<sup>1</sup>), as he now influences, and is influenced by, signal evolution. As we emphasize in this chapter, however, the goldfish preovulatory pheromone mediates more than the simple dyadic spying event depicted in Fig. 23.5a1. The pheromone directly stimulates behavioural and endocrine-testicular responses in more than one male (R) and also induces a distinct response (ovulation) in females (Fig. 23.5a3). In addition, the pheromone indirectly stimulates males (R<sup>2</sup>) via cues released by pheromone-exposed males (Fig. 23.5a4).

In the ancestral condition of predator-induced prey chemical alarm cues, predator (P) attack releases general cues from the originator (O) that can be received both



**Fig. 23.5.** Theoretical evolutionary pathways of the transition between spying and communication networks involving semiochemicals used in reproductive (a) and predator-prey (b) interactions. Thin solid and dashed arrows indicate spying functions; thick, opposed, black and white arrows indicate communicative functions, and large white arrows indicate transitions between proposed stable states. O, originator; S, signaller; R, receiver (r, heterospecific receiver); IE, interceptive eavesdropper; SE, social eavesdropper; P, predator; C, alarm cue. See text for further explanation.

as an alarm cue by conspecific prey (R) and as a feeding cue by secondary predators ( $P^2$ ; Fig. 23.5b1). If interference by secondary predators benefits originators and leads to alarm cue specialization, originators become signallers (S), the secondary predator's role changes ( $P^3$ ), and receiving conspecific prey become interceptive eavesdroppers (IE) in a communication network (Fig. 23.5b2). As with the goldfish pheromone (Fig. 23.5a3,4), predator-induced alarm cues can exert complex effects prior to the evolution of communication. For example, alarm cues are used to

associate risk with stimuli (C) correlated with predation, which later serve as indicators of predation risk (Fig. 23.5b3). This latter system may become elevated to that of a communication network without involvement of a secondary predator if an originator's shoalmates learn to recognize a novel indicator of risk and later alert the surviving originator to the presence of risk through early response to danger (Fig. 23.5b5). In direct relevance to the evolution of eavesdropping, alarm cues can also affect predator-prey interactions indirectly through social facilitation (social spying?) of alarm behaviour both in conspecifics ( $R^2$ ) and in heterospecifics ( $r$ ; Fig. 23.5b4).

If it is reasonable to assume that sex and alarm pheromone communication evolves from spying, as depicted in Figs. 23.1, 23.5a1,2 and 23.5b1,2, then it also seems reasonable to ask whether and how communication networks evolve from spying networks. We, therefore, propose two general scenarios, which differ primarily in the evolutionary origins of eavesdropping. In the first scenario, a simple dyadic communication (Figs. 23.5a2 and 23.5b2) could lead to the evolution of interceptive or social eavesdropping (Ch. 2) if receivers evolve adaptive responses either to the signalling behaviour per se (interceptive eavesdropper (IE): Figs. 23.5a5 and 23.5b6) or to the signalling interaction (social eavesdropper (SE): Figs. 23.5a6 and 23.5b6). In this scenario, where the evolution of communication precedes that of eavesdropping, eavesdropper functions (interceptive and social) are analogous to the various receiver functions in spying networks (Figs. 23.5a3,4 and 23.5b3,4). In the second scenario, incipient eavesdropping arises in spying networks, either as direct (Figs. 23.5a3 and 23.5b4) or indirect (Figs. 23.5a4 and 23.5b4) spying by receivers on originators and is retained as interceptive and social eavesdropping, respectively, following the evolution of communication. In this scenario, receivers in spying networks are homologous to eavesdroppers in communication networks.

In all the scenarios shown in Fig. 23.5, we depict eavesdropping in its proposed initial state: that is, spying via a cue that is not specialized for transmission to eavesdroppers, despite being a signal specialized for information transfer to the *primary target* (Ch. 2). At this early stage, the network functions of eavesdropper and primary target differ in *kind*. However, if subsequent selection by eavesdroppers leads to signal specialization specific to the eavesdropping interaction, and thus forming a communicative relationship between eavesdropper and signaller, functions of eavesdroppers and receivers will come to differ only in *degree*. Studied examples of eavesdropping (Ch. 2) typically appear to involve costs or benefits to signallers that would be expected to modify signal function; consequently, it will be important to determine whether, as has been suggested for sex pheromone function in fish (Fig. 23.5a1), various forms of eavesdropping in communication networks (Figs. 23.5a5,6 and 23.5b2,5,6) can persist as spying. Moreover, it will be important to document covariance in the relative proportions of spying versus

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communicative eavesdropping and the ecological and social factors that lead to the spying–communication transition.

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