

Understanding behavioral responses of fish to pheromones in natural freshwater environments

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Abstract There is an abundance of experimental studies and reviews that describe odorant-mediated behaviors of fish in laboratory microcosms, but research in natural field conditions has received considerably less attention. Fish pheromone studies in laboratory settings can be highly productive and allow for controlled experimental designs; however, laboratory tanks and flumes often cannot replicate all the physical, physiological and social contexts associated with natural environments. Field experiments can be a critical step in affirming and enhancing understanding of laboratory discoveries and often implicate the ecological significance of pheromones employed by fishes. When findings from laboratory experiments have been further tested in field environments, often different and sometimes contradictory conclusions are found. Examples include studies of sea lamprey (*Petromyzon marinus*) mating pheromones and fish alarm substances. Here, we review field research conducted on fish pheromones and alarm substances, highlighting the following topics: (1) contradictory results obtained in laboratory and field experiments, (2) how environmental context and physiological status influences behavior, (3) challenges and constraints of aquatic field research and (4) innovative techniques and experimental designs that advance understanding of fish chemical ecology through field research.

Keywords Pheromone · Fish physiology · Fish behavior · Field study · Laboratory study

Abbreviations

3kPZS	3-Keto petromyzonol sulfate
PZS	Petromyzonol sulfate
ACA	Allocholic acid
17,20 β P	17 α , 20 β -Dihydroxy-4-pregnen-3-one
PGF	Prostaglandin F2 α
PIT	Passive integrated transponder

Introduction

Sensory organs are the filters through which organisms determine the context of their environment and respond accordingly. For humans, it is in our nature to perceive how visual, auditory, gustatory and tactile cues influence animal behavior because it is these senses that we are most familiar with. But since we cannot directly observe the chemical cues animals respond to, given our poorly developed sense of olfaction (Kleerekoper 1969), it often escapes our imagination that chemical communication is the most potent and widely employed mechanism of information transfer in nature (Wyatt 2003; Fisher et al. 2006). Pheromones have diverse roles in modulating vertebrate behavior (Brennan and Zufall 2006). In mammals, pheromones influence ovulation (More 2006), individual recognition (Hurst et al. 2001), mate choice (Spehr et al. 2006) and territorial behavior (Hurst and Beynon 2004). Olfaction is an exquisitely developed sensory capacity in fishes (Kleerekoper 1969), where chemical signals influence behaviors concerning habitat selection (Mathis and Smith 1992), predator avoidance (von Frisch 1938), migration (Hansler and Scholz 1983), maturation (Dulka et al. 1987), mating (Li et al. 2002) and paternal care (Neff 2003). In aquatic environments,

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pheromones often comprise soluble steroids, bile acids or proteins, and commonly communicate a message to a population of conspecifics, sometimes over long distances. Studies of aquatic chemical communication offer unique opportunities to understand how animal behavior and physiology are mediated by chemical signals (Burnard et al. 2008).

Many questions in chemical ecology originate from field observations of animal behavior. Subsequently, scientific inquiry is often moved into the laboratory where experiments are comparable, reproducible, relatively inexpensive, easy to set up and can be conducted year round. Our review of over 400 papers, mostly published between 1960 and the present, found that approximately 80% of studies on chemical communication in fishes and crustaceans have been laboratory exercises. In the cases where laboratory experiments have been extended to the field, results have sometimes been unexpectedly different and contradictory to those obtained in the laboratory. Similar contradictory results between the laboratory and field have been documented in studies of the behavioral and physiological effects of dominance hierarchies in fishes (Sloman and Armstrong 2002; Riley et al. 2005). As a consequence of insufficient space and water, laboratory environments tend to be overly simplistic. For example, flumes and Y-mazes artificially slow and straighten flow (Zimmer et al. 1999) and small aquaria often expose fishes to artificially high concentrations of pheromones for periods of time that would not be encountered in nature (Mackie and Shelton 1972; Pearson 1977). We contend that, because of the inherently artificial nature of laboratory settings, it is highly relevant to understand how pheromones influence fish behavior and physiology in natural environments.

In this review, the need for field confirmation of laboratory results will be highlighted by discussing examples where comparable laboratory and field studies yielded different conclusions. The goal of comparing studies is not to take sides and claim that one study is better than others, but instead to emphasize that the results are indeed different. Possible explanations will be provided as to why laboratory and field results may differ in light of how fish responses to pheromones are revealed only when the appropriate environmental, physiological and social conditions are provided. Experimental constraints of field research will be briefly discussed and examples will be provided as to how obstacles have been overcome. Informative field studies and the innovative techniques and technologies employed will be reviewed and discussed.

Contradictory results obtained in laboratory and field studies

Contradictions between laboratory and field results typically occur when behaviors elicited in the laboratory do not

represent the full suite of chemically mediated behaviors or are not observed in the field. Studies of sea lamprey (*Petromyzon marinus*) pheromones and ostariophysan alarm substances will be used to illustrate the two circumstances by which laboratory and field results may differ.

Sea lamprey exhibit more diverse pheromone-induced behaviors in natural environments

Chemical communication in the sea lamprey has been studied for 30 years and provides many examples to illustrate the utility of field research to understand fish chemical ecology (Li et al. 2007). Larval sea lampreys hatch in streams and feed on algae and detritus. Adults, after parasitically feeding on large fish in the ocean or large lakes, migrate back into streams, spawn and die (Applegate 1950). Pheromones enable adults to efficiently locate suitable spawning streams and mates prior to death (Teeter 1980).

The first indication that sea lampreys use pheromones to coordinate mate finding and reproduction came from field reports of French fishermen who captured high numbers of “ripe” females (ovulated) in traps baited with “roped” males (spermiated) (Fontaine 1938). To confirm these and other reports of female attraction to sexually mature males (Teeter 1980; Adams et al. 1987a, b), a two-choice maze was constructed by Li et al. (2002) on the bank of a natural spawning stream and was supplied with stream water (Fig. 1a). Collectively, maze experiments confirmed that ovulated females did indeed show preference and search responses to the washings of spermiated males (Li et al. 2002; Siefkes et al. 2005). A putative pheromone component, 3-keto petromyzonol sulfate (3kPZS), was extracted from the washings of spermiated males and identified. In the two-choice maze, 3kPZS and spermiated male washings induced statistically similar behaviors in ovulated females. Based on laboratory results, it was concluded that 3kPZS was the male sea lamprey mating pheromone that induced preference and search responses in ovulated females (Li et al. 2002).

To confirm two-choice maze results, experiments were replicated in a natural stream, where an island divides the river into two channels (a natural two-choice maze, Fig. 1b). Contrary to laboratory results, in a stream 3kPZS did not elicit the same preference and search response in ovulated females as spermiated male washings (Siefkes et al. 2005; Johnson et al. 2009). Male washings lured ovulated females upstream over distances up to 250 m to the exact point of odorant release and females remained there for up to an hour. Synthesized 3kPZS also lured females upstream 250 m, but females typically entered within 1 m of the source, where they briefly paused and then continued moving upstream (Siefkes et al. 2005; Johnson et al. 2009).

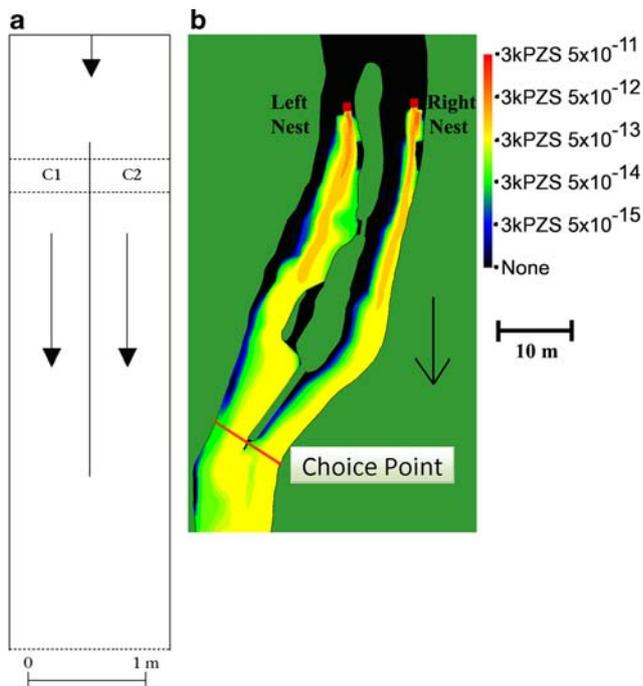


Fig. 1 Comparison of laboratory and field bioassays used to study sea lamprey pheromones. **a** Water is pumped into the two-choice maze at the upper arrow and flows down to where the maze is split into two chambers. A test odorant is added to the maze at C1 or C2, and the time a sea lamprey spends on each side of the maze before and after odorant application is recorded. The functional length and width of the maze is 4.27 by 1.22 m. Reprinted from Siefkes et al. (2005), with permission from Elsevier. **b** The Ocqueoc River MI, USA is divided into two channels by an island. Behavioral responses of sea lampreys to pheromones can be tested over hundreds of meters by applying pheromones to one channel and control odorant to the other to create a natural two-choice maze. Arrow indicates flow direction and test odorants were applied in the red square in each channel. Application of rhodamine dye was used to determine the distribution and concentration of synthesized male sea lamprey mating pheromone (3kPZS) originating from the location of each odorant application

Results from in-stream experiments were inconsistent with the conclusion derived from maze experiments and demonstrated that pheromone components other than 3kPZS are needed to elicit near source attraction and retention in ovulated females.

In addition to mating pheromones, adult sea lampreys have also been hypothesized to use bile acid migratory pheromones released by conspecific larvae to locate suitable spawning streams (Bjerselius et al. 2000). Evidence of sea lamprey migratory pheromones originated from field observations that numbers of migratory adults entering streams treated the previous year with the lampricide 3-trifluoromethyl-4-nitrophenol (kills about 95% of larvae) drop drastically (Moore and Schleen 1980). To confirm field observations, Teeter (1980) showed in a maze that migratory sea lampreys were attracted to larval lamprey washings. In 1995, Li et al. found petromyzonol sulfate (PZS) to be highly stimulatory to the olfactory organ of sea

lampreys as measured by an electro-olfactogram. In 2000, Bjerselius et al. tested PZS as well as allocholic acid (ACA), another putative migratory pheromone, in a two-choice maze and concluded that PZS and ACA were important components of the migratory pheromone. A year later, however, Vrieze and Sorensen (2001) concluded from maze studies that little of the activity found in the migratory pheromone can be explained by PZS and ACA. Instead, Vrieze and Sorensen reported that migrating lampreys strongly prefer blank stream water (without larval pheromones) to the water of Lake Huron. Unfortunately, all prior experiments were conducted in the water of Lake Huron or well water. Most recently, it has been reported that two newly identified bile acids, petromyzonamine disulfate (PADS) and petromyzosterol disulfate (PSDS), along with PZS, constitute the sea lamprey migratory pheromone (Sorensen et al. 2005). Field confirmation that any of the putative pheromones elicit behavioral responses in nature is still pending (Wagner et al. 2009).

Alarm substances elicit greater responses in the laboratory than in the field

Laboratory and field studies of ostariophysan alarm substances (Schreckstoff) have been among the most controversial in chemical ecology. The term Schreckstoff was coined by von Frisch in 1938 who, using field observations, described an “alarm pheromone” released by injured European minnows (*Phoxinus phoxinus*) that elicited fright responses in conspecifics (von Frisch 1938, 1941). Since von Frisch’s seminal work, most studies have been moved into the laboratory and over 100 published aquaria studies concerning Schreckstoff collectively show that some ostariophysan fishes contain epidermal club cells that when ruptured release an odorant, which induces alarm responses in conspecifics and some heterospecifics (Brown et al. 1995; Mikheev et al. 2006; Chivers et al. 2007).

The function of Schreckstoff was not debated in writing until Magurran et al. (1996) used an underwater camera to observe the behavior of wild European minnows before and after exposure to conspecific skin extract (Schreckstoff) and muscle extract (control). Different from the report by von Frisch (1938) and laboratory studies of other authors, Schreckstoff failed to elicit stereotypical alarm responses such as dashing, hiding, freezing or leaving the observation area. The authors postulated that alarm responses to Schreckstoff may be artifacts of the confined and satiated condition of laboratory fishes. Furthermore, in the wild, responses to Schreckstoff may be related to the relative threat of predation. Magurran et al. (1996) stated that because the alarm response of European minnows to Schreckstoff was contingent on “other factors”, it was not a “pheromone”. However, published literature does not

suggest that pheromones must elicit response in all contexts and presented no examples of pheromones or alarm substances that elicited a behavioral response in all conspecifics in all environments. Behavioral and physiological responses of fish to odorants are modulated more by context and the ability to use sensory modalities other than olfaction, rather than by specific pheromones (Carolsfeld et al. 1997b; Smith 1997; Bjerselius et al. 2000; Fraser and Stacey 2002; Golub et al. 2005). Results of laboratory and field experiments provide sometimes different, yet informative, perspectives for understanding pheromone function.

Environmental, social, and physiological context modulate the expression of chemically mediated behaviors

Environmental context

Physical structure in the aquatic environment

Structural complexity and composition of the aquatic environment can influence chemically mediated behaviors of fishes. Golub and Brown (2003) documented differences in pumpkinseed (*Lepomis gibbosus*) response to alarm substances in different habitat complexities. In weedless environments, pumpkinseeds showed less vigorous responses to alarm substances than in weedy environments. Golub and Brown (2003) illustrate that multi-modal cues provide more information than a single cue. Pumpkinseeds may not show alarm responses in weedless environments because they are better able to use visual cues, which more effectively convey the location of potential danger. Substrate type also strongly influences chemically mediated behaviors in fishes, especially if reproduction is substrate dependent. The sound of female redd cutting triggered an increase in $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one ($17,20\beta$ P) and of expressible milt in male Atlantic salmon parr (*Salmo salar*) in laboratory environments (Moore and Waring 1999). In the two-choice maze used for sea lamprey mating pheromone research, a potential flaw in the design was that it did not contain rocky substrate needed for spawning (Applegate 1950; Li et al. 2002). These examples suggest that the physical environment often determines the degree of response elicited by odorants in fishes because when, for example, vision is obfuscated by attributes of the environment, information from the remaining sense of olfaction becomes more influential in modulating behavior.

Water source and chemistry

Water clarity, chemistry and source can influence responsiveness of fishes to chemical cues. Fishes often distinguish

and prefer water originating from habitat types needed for essential life history events. Migratory sea lampreys strongly prefer stream water (pheromone free) compared to water from Lake Huron, and responses to migratory pheromones are more potent when tested in stream water (Vrieze and Sorensen 2001). Water clarity also affects fish responses to alarm substances, where stronger responses occur in turbid water than in clear water, presumably because turbidity obstructs visual cues (Hartman 2000).

Where studied, fish pheromones and alarm substances are more stable and have higher detection thresholds in water with slightly basic pH (Brown et al. 2001a, 2002a; Bhatt et al. 2002; Leduc et al. 2004; Heuschele and Candolin 2007). Fathead minnow (*Pimephales promelas*) responses to alarm substances in laboratory conditions are abolished at a pH less than 6.0 (Brown et al. 2000, 2002a). The mechanism by which basic conditions enhance responsiveness is still unresolved. One possibility is that basic water shifts the ionic phase of the chemical cue, making its new three-dimensional structure more accessible to olfactory receptors (Heuschele and Candolin 2007). Alternatively, acidic water may shift the chemical structure of odorants through protonation, rendering them unavailable to receptor sites as hypothesized for putative nitrogen oxide alarm substances (Brown et al. 2000; Leduc et al. 2004). Acidification may also be stressful to fishes, thereby limiting responses to alarm substances.

The ability of fishes to detect pheromones can be abolished by humic acid, pesticides, heavy metals and anesthetics (Lurling and Scheffer 2007). In goldfish (*Carassius auratus*), humic acid at concentrations between 1 and 1,000 mg/L have been shown to reduce the availability of steroidal mating pheromones in the olfactory epithelium (Hubbard et al. 2002), possibly because they become absorbed into the core of humic acid hydrophobic microvesicles (Hubbard et al. 2002; Mesquita et al. 2003). In eutrophic streams of northern Mexico, it is hypothesized that high concentrations of humic acid affect the ability of two swordtail fishes (*Xiphophorus birchmanni* and *Xiphophorus malinche*) to discriminate between species by olfaction, therefore leading to the recent hybridization of these species (Fisher et al. 2006).

Other olfactory disruptants measured at physiologically relevant concentrations in natural waters include copper (heavy metal), cadmium (heavy metal, Scott et al. 2003; Carreau and Pyle 2005), atrazine, simazin (herbicides, Moore and Waring 1998; Moore and Lower 2001), diazinon (insecticide, Scholz et al. 2000) and cypermethrin (pyrethroid insecticide, Moore and Waring 2001; Jaensson et al. 2007). Researchers may unintentionally reduce olfactory sense in experimental fishes by using anesthetics such as quinaldine, phenoxyethanol or 3-aminobenzoic acid ethyl ester (MS-222) (Losey and Hugie 1994).

Hydrodynamics

Responses of fishes to chemical cues can vary dramatically depending on the rates of advection, coefficients of turbulent mixing and boundary layer dynamics because these factors determine the spatial and temporal distribution of odorant plumes and the frequency of encounters between odorants and olfactory receptors. Few studies have evaluated how hydrodynamics influence behavioral responses of fish to pheromones. Sherman and Moore (2001) showed that whether water is lentic or lotic can directly influence the ability of brown bullheads (*Ameiurus nebulosus*) to locate fish gelatin. The sea lamprey is another example where one environmental condition modulates the response to olfactory cues. In this species, responsiveness to migratory pheromones has been shown to be dependent on water velocity, where flow direction may be used to orient toward sources of pheromones via chemically mediated rheotaxis (Bjerselius et al. 2000). The absence of flow “turns off” the response to the odorant, much like the addition of vision-reduced responses to alarm substances in pumpkinseed (Golub et al. 2005).

In comparison to the few studies on fishes, orientation responses of crustaceans in different flow environments have been well investigated. Weissburg and Zimmer-Faust (1994) showed that blue crabs (*Callinectes sapidus*) orient to food odors efficiently when boundary layer flows are smooth than when rough, at which time crabs move more slowly and make more frequent stops. In direct contrast, crayfish (*Onconectes rusticus*) orient to food odors more efficiently on cobble substrate (roughness Reynolds number of 382) than on sand (roughness Reynolds number of 1.93; Moore and Grills 1999). How an odorant is applied to the hydrodynamic environment also influences crustacean orientation efficiency (Keller and Weissburg 2004). The hydrodynamics of experimental systems should be controlled and described so that results may be comparable among studies (Moore and Grills 1999).

Temporal variation in responses to chemical cues

Aquatic organisms may only respond to chemical cues during certain times of the day. Migratory adult sea lamprey are nocturnal and respond to migratory pheromones at night (Bjerselius et al. 2000). However, sexually mature sea lampreys are arrhythmic (Applegate 1950); notably, mature females respond to mating pheromones night and day (Johnson et al. 2005). A goldfish female priming pheromone, 17,20 β P, robustly induces sexual arousal in males in the early morning when females are likely to release 17,20, β P. However, the same 17,20, β P in the afternoon induces lower levels of arousal in the males (Defraipont and Sorensen 1993).

Social context, experience and learning

Social environment is an important context that can influence responses of fishes to pheromones (Ferrari et al. 2005). In goldfish, the effects of social context on male response to female priming pheromones 17,20 β P and prostaglandin F2 α (PGF) have been investigated. When in social isolation, the male endocrine system is only primed by exposure to 17,20 β P (Fraser and Stacey 2002). When males are able to socially interact with females, the male endocrine system is primed by PGF (Sorensen et al. 1989).

Experience and learning also alter behavioral responses of fishes to alarm substances. Several fish species exhibit learned predator recognition to alarm substances. Simultaneous exposure of an individual to a novel odorant and a stressful event, like chasing with a net, causes the individual to associate the odorant with danger (Chivers et al. 1995; Kelley and Magurran 2003). Hatchery reared fish have been trained to recognize odors of natural predators, but the efficacy of this technique to reduce mortality of stocked fish is yet to be demonstrated (Brown and Smith 1998; Mirza and Chivers 2000). Zebrafish (*Brachydanio rerio*) have even been trained to associate red light with predation threat by simultaneously exposing them to alarm substances and red light (Hall and Suboski 1995a, b). Recognition of odorants can last up to a month and have been demonstrated in field contexts (Pollock et al. 2003).

Physiological and developmental status

Often influenced by environmental context, the physiological status of an animal ultimately determines whether a behavioral response is elicited by reproductive pheromones and alarm substances. Responsiveness to odorants can be influenced by the collinear effects of age, size, sex, maturity (Dulka et al. 1987; Li et al. 2002), hormone concentrations (Cardwell et al. 1995; Carolsfeld et al. 1997a; Yambe and Yamazaki 2001; Bhatt et al. 2002), stress (Scott et al. 1994; Carolsfeld et al. 1997a) and hunger (Smith 1981; Brown and Smith 1996; Chivers et al. 2000).

Size and hunger influences responsiveness to alarm substances

Some species undergo an ontogenetic shift where alarm substance responses are only elicited in young age groups, likely due to trade-offs between foraging and predation risk (Mirza and Chivers 2002; Golub and Brown 2003; Harvey and Brown 2004; Golub et al. 2005). Small largemouth bass (<50 mm) (*Micropterus salmoides*) respond with anti-predator responses to heterospecific alarm substances, but large-sized bass (>50 mm) respond with feeding behaviors (Brown et al. 2001b, 2002b). Hunger also mediates the

degree of antipredator response when fish are exposed to alarm substances. Smith (1981) found that Iowa darters (*Etheostoma exile*), after fasting for 12 h, responded to a mixture of food and alarm substances with a feeding response, but darters responded to the same odorant with an alarm response when satiated. Similarly, alarm responses in fathead minnows and reticulate sculpins (*Cottus perplexus*) are abolished after 24 h of fasting (Brown and Smith 1996). The hunger of experimental fish may explain the contradictory results obtained in laboratory and field studies of alarm substances (Magurran et al. 1996), wherein the laboratory fish are often fed to satiation, but in the wild food resources may be limited.

Age, sex and maturity

Studies on the sea lamprey clearly illustrate how pheromone-induced behaviors are dependent on age, sex and maturity. When adults migrate upstream, male and female olfactory systems are highly sensitive to migratory pheromones released by larval sea lamprey and show preference responses to migratory pheromones (Sorensen and Vrieze 2003a, b). When sexually mature, lamprey responses to migratory pheromones diminish (Bjerselius et al. 2000); but at that time, spermiated males construct spawning nests and release mating pheromones that are highly attractive to ovulated females (Siefkes et al. 2005). Similar sex and maturity effects have been reported in the goldfish, where females sequentially release mixtures of pheromones throughout the ovulation cycle to prime males and induce courtship behaviors, a subject that has been reviewed extensively (Sorensen 1992; Sorensen et al. 1998; Kobayashi et al. 2002; Stacey 2003; Stacey et al. 2003; Sorensen and Stacey 2004).

In fishes with alternative mating strategies, dominant and sneaker males may differ in pheromone release and response. Dominant male black gobies (*Gobius niger*) respond aggressively to the ejaculate of other dominant males, but not to the ejaculate of sneaker males, likely because sneaker male ejaculate is pheromonally inconspicuous (Locatello et al. 2002). In some cases, however, sneaker males can detect and respond to conspecific pheromones. Yambe et al. (2006) used “jack” masu salmon (*Oncorhynchus masou*) as bioassay subjects to identify female mating pheromones, because small jacks were easier to work with in the laboratory and yet responded to pheromones.

Responsiveness to mating pheromones has been associated to increases in plasma sex steroid concentration in several species. Carolsfeld et al. (1997b) showed in Pacific herring (*Clupea pallasii*) that individuals with higher sex steroid concentrations were more likely to respond to migratory pheromones. Immature male parr of masu

salmon and rainbow trout (*Oncorhynchus mykiss*), which do not naturally respond to female priming and mating pheromones, respond when treated with methyltestosterone (Yambe and Yamazaki 2001; Yambe et al. 2003). Similarly, an injection of androgens into male *Barilius bendelisis* increased the responsiveness to female mating pheromones by activating male olfactory receptors (Bhatt et al. 2002).

Stress

Handling fish or exposing them to sudden changes in environmental conditions typically induces a stress response. For example, transport of brown trout (*Salmo trutta*) (Pickering et al. 1987) and rainbow trout (Pottinger 1992; Scott et al. 1994) from the field to the laboratory has been shown to be highly stressful. Stressed fish may modify their behavioral responses to mating pheromones, because a sudden increase in cortisol lowers sex steroid concentrations. A notable exception has been described in Pacific herring, where a shallow water stressor induces vigorous responses to spawning pheromones (Carolsfeld et al. 1997b), perhaps because they spawn on shallow rock reefs.

Available methodologies and variables to consider when designing field studies

Observation of animals

Fish responses to chemical cues can be recorded by research personnel who observe behavior (Johnsen and Hasler 1980), telemetry coupled with observed behavior (Johnson et al. 2005, 2006; Siefkes et al. 2005), snorkel surveys (Neff 2003; Golub et al. 2005) and underwater cameras (Magurran et al. 1996; Wisenden et al. 2004b; Friesen and Chivers 2006). Disadvantages of manually tracking individual animals are that some animals may be lost, many observers are needed, observations times are short, and behaviors are not video recorded and thus cannot be reviewed. Snorkel surveys require many observers, and care must be taken not to disturb natural fish behavior. Field studies using underwater camera technologies, although with limited viewing space, have enabled researchers to understand the context dependence of alarm substance responses.

Surrogate measures of behavior are collected when it is not feasible to directly observe fishes. Examples include (1) the number of fish (or lack of fish) captured in traps baited with alarm substances (Mathis and Smith 1992; Wisenden et al. 1994, 2004a; Chivers et al. 1995; Mathis et al. 1995; Mirza and Chivers 2001; Pollock et al. 2003, 2005; Wisenden 2008) and (2) monitoring in-stream movements of sea

lampreys tagged with passive integrated transponders (PIT tags and antennas, Oregon RFID; Wagner et al. 2006, 2009; Johnson et al. 2009). Surrogate measures are limited where, for example, PIT tag data can only inform about the speed and direction of movement of a fish as it passes a stream location, but cannot reveal if fine-scale pheromone-induced behaviors were elicited such as nest building or courtship.

Although never encountered in our review of fish pheromone field studies, new acoustic telemetry technology could allow the three-dimensional movements of fishes to be plotted, revealing orientation responses to pheromones at lake and river scales. With determination, creativity and new technology, researchers can overcome obstacles of observing animals in field studies.

Experimental animals

Fishes naturally present in the environment have been used as test subjects in most field studies reviewed (Finelli et al. 2000; Brown et al. 2001b; Carton and Montgomery 2003). Using wild animals can limit experimental design, because it is difficult to identify individuals, obtain physiological data (size, age, sex, maturity), evaluate social interactions, control for past experiences, insure sample size will be adequate (Scholz et al. 2000) and determine whether the same animal was tested more than once (pseudo-replication). Establishing experimental sites hundreds of meters apart reduced the chance of pseudo-replication in a study conducted by Brown et al. (2001b). Zimmer et al. (1999) evaluated the likelihood of pseudo-replication by estimating the density of the experimental species in the field site using mark-recapture techniques.

To further reduce the chance of pseudo-replication, many studies relied on tagging and releasing captured animals into study sites (Siefkes et al. 2005; Johnson et al. 2006, 2009; Wagner et al. 2006). Advantages of releasing test subjects are that a known number of uniquely marked animals, of specific sex and maturity, can be released from a known location and time. Disadvantages of introducing animals are that test subjects may be stressed prior to experimentation and additional holding facilities and labor are required. If groups of animals are released, one must determine if individuals behaved independently within the group and if individuals tested on different trial dates responded differently (Siefkes et al. 2005; Johnson et al. 2006, 2009).

Unknown pheromone identity limits design of field experiments

A significant constraint for field research is the unknown chemical identity of most fish pheromones and hence a lack of synthesized pheromones. Field studies often require large quantities of pheromone and synthesis of known

pheromones becomes very important. When pheromone identity is not known, active components must be collected or extracted from fish, and the concentration of the active component in extracts cannot be determined (Friesen and Chivers 2006). Recent advances in the extraction (Fine et al. 2006), identification, measurement and synthesis of sea lamprey migratory and mating pheromones (Li et al. 2002; Yun et al. 2003; Sorensen et al. 2005; Dvornikovs et al. 2006; Hoye et al. 2007; Wagner et al. 2009) have allowed large-scale field experiments for testing the efficacy of pheromones to control sea lamprey (Johnson et al. 2005, 2006, 2009; Siefkes et al. 2005; Wagner et al. 2006).

Similar chemistry techniques used to identify sea lamprey pheromones have been used to identify mating pheromones in the masu salmon (Yambe et al. 2006) and are becoming more widely used among chemical ecologists. New advances in analytical chemistry techniques will soon allow for quantification of known mating and migratory pheromone components directly from lake and stream water (Scott and Ellis 2007). Confirming pheromone application rates and determining background concentrations of pheromones in natural systems will greatly increase experimental design options.

Distribution of chemical cues in natural systems

It was evident from numerous studies that by integrating behavioral data, odor plume data and turbulence calculations, chemo-orientation mechanisms of aquatic organisms could be revealed. The distribution of crustacean food odorants in field conditions have been thoroughly described at small spatial scales using dyes and electrochemical tracers such as dopamine (Zimmer-Faust et al. 1995; Finelli et al. 1999, 2000; Zimmer et al. 1999). Zimmer-Faust et al. (1995) described the attraction of predatory blue crabs to food odorants originating from hard crabs (*Mercenaria mercenaria*) in a tidal creek. They recorded blue crab movements using mounted video cameras and digitized movements of crabs that approached the food odorant. Odor plume dynamics were traced by mixing fluorescein (dye tracer) and dopamine (electrochemical tracer) with the odorant. Time-averaged fluorescein concentrations were determined at sampling locations throughout the field site. At the same sampling locations, instantaneous dopamine concentrations were recorded. Flow characteristics were described with an electromagnetic flow meter, and shear velocities and Reynolds numbers were calculated (Denny 1988, 1993). Estimates of sea lamprey mating pheromone concentration and distribution over stream distances of hundreds of meters have been obtained with rhodamine dye tests (Fig. 1b), but they only displayed a static picture of odorant distribution and did not provide data on the

variability and filamentous nature of the pheromone plume, which limits insight into chemo-orientation mechanisms.

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References

- Adams MA, Teeter JH, Katz Y, Johnsen PB (1987a) Reproductive pheromones of the landlocked sea lamprey (*Petromyzon marinus*)—studies on urinary steroids. *Ann NY Acad Sci* 510:148–151
- Adams MA, Teeter JH, Katz Y, Johnsen PB (1987b) Sex-pheromones of the sea lamprey (*Petromyzon marinus*)—steroid studies. *J Chem Ecol* 13:387–395
- Applegate VC (1950) The natural history of the sea lamprey in Michigan. US Department of Interior Fish & Wildlife Service, Special Scientific Report Fisheries, Washington, DC
- Bhatt JP, Kandwal JS, Nautiyal R (2002) Water temperature and pH influence olfactory sensitivity to pre-ovulatory and post-ovulatory ovarian pheromones in male *Barilius bendelisis*. *J Biosci* 27:273–281
- Bjerselius R, Li W, Teeter JH, Seelye JG, Johnsen PB, Maniak PJ, Grant GC, Polkinghorne CN, Sorensen PW (2000) Direct behavioral evidence that unique bile acids released by larval sea lamprey (*Petromyzon marinus*) function as a migratory pheromone. *Can J Fish Aquat Sci* 57:557–569
- Brennan PA, Zufall F (2006) Pheromone communication in vertebrates. *Nature* 444:308–315
- Brown GE, Smith RJF (1996) Foraging trade-offs in fathead minnows (*Pimephales promelas*): acquired predator recognition in the absence of an alarm response. *Ethology* 102:776–785
- Brown GE, Smith RJF (1998) Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery-reared fish to recognize chemical cues of a predator. *Can J Fish Aquat Sci* 55:611–617
- Brown GE, Chivers DP, Smith RJF (1995) Fathead minnows avoid conspecific and heterospecific alarm pheromones in the feces of northern pike. *J Fish Biol* 47:387–393
- Brown GE, Adrian JC, Smyth E, Leet H, Brennan S (2000) Ostariophysan alarm pheromones: laboratory and field tests of the functional significance of nitrogen oxides. *J Chem Ecol* 26:139–154
- Brown GE, Adrian JC, Patton T, Chivers DP (2001a) Fathead minnows learn to recognize predator odour when exposed to concentrations of artificial alarm pheromone below their behavioural-response threshold. *Can J Zool* 79:2239–2245
- Brown GE, LeBlanc VJ, Porter LE (2001b) Ontogenetic changes in the response of largemouth bass (*Micropterus salmoides*, Centrarchidae, Perciformes) to heterospecific alarm pheromones. *Ethology* 107:401–414
- Brown GE, Adrian JC, Lewis MC, Tower JM (2002a) The effects of reduced pH on chemical alarm signaling in ostariophysan fishes. *Can J Fish Aquat Sci* 59:1331–1338
- Brown GE, Gershaneck DL, Plata DL, Golub JL (2002b) Ontogenetic changes in response to heterospecific alarm cues by juvenile largemouth bass are phenotypically plastic. *Behaviour* 139:913–927
- Burnard D, Gozlan RE, Griffiths SW (2008) The role of pheromones in freshwater fishes. *J Fish Biol* 73:1–16
- Cardwell JR, Stacey NE, Tan ESP, Mcadam DSO, Lang SLC (1995) Androgen increases olfactory receptor response to a vertebrate sex-pheromone. *J Comp Physiol A* 176:55–61
- Carolsfeld J, Scott AP, Sherwood NM (1997a) Pheromone-induced spawning of Pacific herring. II. Plasma steroids distinctive to fish responsive to spawning pheromone. *Horm Behav* 31:269–276
- Carolsfeld J, Tester M, Kreiberg H, Sherwood NM (1997b) Pheromone-induced spawning of Pacific herring. I. Behavioral characterization. *Horm Behav* 31:256–268
- Carreau ND, Pyle GG (2005) Effect of copper exposure during embryonic development on chemosensory function of juvenile fathead minnows (*Pimephales promelas*). *Ecotox Environ Safe* 61:1–6
- Carton AG, Montgomery JC (2003) Evidence of a rheotactic component in the odour search behaviour of freshwater eels. *J Fish Biol* 62:501–516
- Chivers DP, Wisenden BD, Smith RJF (1995) The role of experience in the response of fathead minnows (*Pimephales promelas*) to skin extract of Iowa darters (*Etheostoma exile*). *Behaviour* 132:665–674
- Chivers DP, Puttlitz MH, Blaustein AR (2000) Chemical alarm signaling by reticulate sculpins (*Cottus perplexus*). *Environ Biol Fish* 57:347–352
- Chivers DP, Wisenden BD, Hindman CJ, Michalak TA, Kusch RC, Kaminskyj SGW, Jack KL, Ferrari MCO, Pollock RJ, Halbgewachs CF, Pollock MS, Alemadi S, James CT, Savaloja RK, Goater CP, Corwin A, Mirza RS, Kiesecker JM, Brown GE, Adrian JC, Krone PH, Blaustein AR, Mathis A (2007) Epidermal ‘alarm substance’ cells of fishes are maintained by non-alarm functions: possible defense against pathogens, parasites and UVB radiation. *Proc R Soc Lond B* 274:2611–2619
- Defraipont M, Sorensen PW (1993) Exposure to the pheromone 17-alpha, 20-beta-dihydroxy-4-pregnen-3-one enhances the behavioral spawning success, sperm production and sperm motility of male goldfish. *Anim Behav* 46:245–256
- Denny MW (1988) Biology and the mechanics of the wave-swept environment. Princeton University Press, Princeton
- Denny MW (1993) Air and water. Princeton University Press, Princeton
- Dulka JG, Stacey NE, Sorensen PW, Vanderkraak GJ (1987) A steroid sex-pheromone synchronizes male–female spawning readiness in goldfish. *Nature* 325:251–253
- Dvornikovs V, Fine JM, Hoye TR, Jeffrey CS, Shao F, Wang J, Vrieze LA, Anderson KR, Sorensen PW (2006) Isolation and structure elucidation of the sea lamprey migratory pheromone. *Chem Senses* 31:A82–A83
- Ferrari MCO, Trowell JJ, Brown GE, Chivers DP (2005) The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. *Anim Behav* 70:777–784
- Fine JM, Sisler SP, Vrieze LA, Swink WD, Sorensen PW (2006) A practical method for obtaining useful quantities of pheromones from sea lamprey and other fishes for identification and control. *J Great Lakes Res* 32:832–838
- Finelli CM, Pentcheff ND, Zimmer-Faust RK, Wetthey DS (1999) Odor transport in turbulent flows: constraints on animal navigation. *Limnol Oceanogr* 44:1056–1071
- Finelli CM, Pentcheff ND, Zimmer RK, Wetthey DS (2000) Physical constraints on ecological processes: a field test of odor-mediated foraging. *Ecology* 81:784–797
- Fisher HS, Wong BBM, Rosenthal GG (2006) Alteration of the chemical environment disrupts communication in a freshwater fish. *P Roy Soc B-Biol Sci* 273:1187–1193
- Fontaine M (1938) La lamproie marine. Sa peche et son importance economique. *B Sco Ocean Fr* 17:1681–1687
- Fraser EJ, Stacey NE (2002) Isolation increases milt production in goldfish. *J Exp Biol* 293:511–524
- Friesen RG, Chivers DP (2006) Underwater video reveals strong avoidance of chemical alarm cues by prey fishes. *Ethology* 112:339–345

- Golub JL, Brown GE (2003) Are all signals the same? Ontogenetic change in the response to conspecific and heterospecific chemical alarm signals by juvenile green sunfish (*Lepomis cyanellus*). *Behav Ecol Sociobiol* 54:113–118
- Golub JL, Vermette V, Brown GE (2005) Response to conspecific and heterospecific alarm cues by pumpkinseeds in simple and complex habitats: field verification of an ontogenetic shift. *J Fish Biol* 66:1073–1081
- Hall D, Suboski MD (1995a) Sensory preconditioning and 2nd-order conditioning of alarm reactions in zebra danio fish (*Brachydanio rerio*). *J Comp Psychol* 109:76–84
- Hall D, Suboski MD (1995b) Visual and olfactory stimuli in learned release of alarm reactions by zebra danio fish (*Brachydanio rerio*). *Neurobiol Learn Memory* 63:229–240
- Hansler AD, Scholz AT (1983) Olfactory imprinting and homing in salmon. Springer, Berlin
- Hartman EJ (2000) Sensory compensation and the detection of predators: the interaction between chemical and visual information. *P Roy Soc B-Biol Sci* 267:571–575
- Harvey MC, Brown GE (2004) Dine or dash?: Ontogenetic shift in the response of yellow perch to conspecific alarm cues. *Environ Biol Fish* 70:345–352
- Heuschele J, Candolin U (2007) An increase in pH boosts olfactory communication in sticklebacks. *Biology Lett* 3:411–413
- Hoye TR, Dvornikovs V, Fine JM, Anderson KR, Jeffrey CS, Muddiman DC, Shao F, Sorensen PW, Wang J (2007) Details of the structure determination of the sulfated steroids PSDS and PADS: new components of the sea lamprey (*Petromyzon marinus*) migratory pheromone. *J Org Chem* 72:7544–7550
- Hubbard PC, Barata EN, Canario AV (2002) Possible disruption of pheromonal communication by humic acid in the goldfish, *Carassius auratus*. *Aquat Toxicol* 60:169–183
- Hurst J, Beynon R (2004) Scent wars: the chemobiology of competitive signaling in mice. *BioEssays* 26:1288–1298
- Hurst J, Payne CE, Nevison CM, Marie AD, Humphries RE, Robertson DHL, Cavagioni A, Beynon TB (2001) Individual recognition in mice mediated by major urinary proteins. *Nature* 414:631–634
- Jaansson A, Scott AP, Moore A, Kylin H, Olsen KH (2007) Effects of a pyrethroid pesticide on endocrine responses to female odours and reproductive behaviour in male parr of brown trout (*Salmo trutta* L.). *Aquat Toxicol* 81:1–9
- Johnsen PB, Hasler AD (1980) The use of chemical cues in the upstream migration of coho salmon, *Oncorhynchus kisutch* Walbaum. *J Fish Biol* 17:67–73
- Johnson NS, Siefkes MJ, Li W (2005) Capture of ovulating female sea lampreys in traps baited with spermiating male sea lampreys. *N Am J Fish Manage* 25:67–72
- Johnson NS, Luehring MA, Siefkes MJ, Li W (2006) Mating pheromone reception and induced behavior in ovulating female sea lampreys. *N Am J Fish Manage* 26:88–96
- Johnson NS, Yun S-S, Thompson HT, Brant CB, Li W (2009) A synthesized pheromone induces upstream movement in female sea lampreys and summons them into traps. *P Natl Acad Sci USA* 106:1021–1026
- Keller TA, Weissburg MJ (2004) Effects of odor flux and pulse rate on chemosensory tracking in turbulent odor plumes by the blue crab, *Callinectes sapidus*. *Biol Bull* 207:44–55
- Kelley JL, Magurran AE (2003) Learned predator recognition and anti-predator responses in fishes. *Fish Fish* 4:216–226
- Kleerekoper H (1969) Olfaction in fishes. Indiana University Press, Bloomington
- Kobayashi M, Sorensen PW, Stacey NE (2002) Hormonal and pheromonal control of spawning behavior in the goldfish. *Fish Physiol Biochem* 26:71–84
- Leduc A, Kelly JM, Brown GE (2004) Detection of conspecific alarm cues by juvenile salmonids under neutral and weakly acidic conditions: laboratory and field tests. *Oecologia* 139:318–324
- Li W, Sorensen PW, Gallaher DD (1995) The olfactory system of migratory adult sea lamprey (*Petromyzon marinus*) is specifically and acutely sensitive to unique bile acids released by conspecific larvae. *J Gen Physiol* 105:569–587
- Li W, Scott AP, Siefkes MJ, Yan H, Liu Q, Yun S-S, Gage DA (2002) Bile acid secreted by male sea lamprey that acts as a sex pheromone. *Science* 296:138–141
- Li W, Twohey M, Jones M, Wagner M (2007) Research to guide use of pheromones to control sea lamprey. *J Great Lakes Res* 33(2):70–86
- Locatello L, Mazzoldi C, Rasotto MB (2002) Ejaculate of sneaker males is pheromonally inconspicuous in the black goby, *Gobius niger* (Teleostei, Gobiidae). *J Exp Zool* 293:601–605
- Lossy GS, Hugie DM (1994) Prior anesthesia impairs a chemically mediated fright response in a gobiid fish. *J Chem Ecol* 20:1877–1883
- Lurling M, Scheffer M (2007) Info-disruption: pollution and the transfer of chemical information between organisms. *Trends Ecol Evol* 22:374–379
- Mackie AM, Shelton RGJ (1972) A whole-animal bioassay for the determination of food attractants of the lobster *Homarus gammarus*. *Mar Biol* 14:217–221
- Magurran AE, Irving PW, Henderson PA (1996) Is there a fish alarm pheromone? A wild study and critique. *P Roy Soc B-Biol Sci* 263:1551–1556
- Mathis A, Smith RJF (1992) Avoidance of areas marked with a chemical alarm substance by fathead minnows (*Pimephales promelas*) in a natural habitat. *Can J Zool* 70:1473–1476
- Mathis A, Chivers DP, Smith RJF (1995) Chemical alarm signals—predator deterrents or predator attractants. *Am Nat* 145:994–1005
- Mesquita RM, Canario AV, Melo E (2003) Partition of fish pheromones between water and aggregates of humic acids. Consequences for sexual signaling. *Environ Sci Technol* 37: 742–746
- Mikheev VN, Wanzenbock J, Pasternak AF (2006) Effects of predator-induced visual and olfactory cues on 0+ perch (*Perca fluviatilis* L.) foraging behaviour. *Ecol Freshw Fish* 15:111–117
- Mirza RS, Chivers DP (2000) Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. *Can J Zool* 78:2198–2208
- Mirza RS, Chivers DP (2001) Chemical alarm signals enhance survival of brook charr (*Salvelinus fontinalis*) during encounters with predatory chain pickerel (*Esox niger*). *Ethology* 107:989–1005
- Mirza RS, Chivers DP (2002) Brook char (*Salvelinus fontinalis*) can differentiate chemical alarm cues produced by different age/size classes of conspecifics. *J Chem Ecol* 28:555–564
- Moore PA, Grills JL (1999) Chemical orientation to food by the crayfish *Orconectes rusticus*: influence of hydrodynamics. *Anim Behav* 58:953–963
- Moore A, Lower N (2001) The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. *Comp Biochem Phys B* 129:269–276
- Moore HH, Schleen IP (1980) Changes in spawning runs of sea lamprey (*Petromyzon-marinus*) in selected streams of Lake Superior after chemical control. *Can J Fish Aquat Sci* 37:1851–1860
- Moore A, Waring CP (1998) Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. *Pestic Biochem Phys* 62:41–50
- Moore A, Waring CP (1999) Reproductive priming in mature male Atlantic salmon parr exposed to the sound of redd cutting. *J Fish Biol* 55:884–887
- Moore A, Waring CP (2001) The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). *Aquat Toxicol* 52:1–12

- More L (2006) Mouse major urinary proteins trigger ovulation via the vomeronasal organ. *Chem Senses* 31:393–401
- Neff BD (2003) Decisions about parental care in response to perceived paternity. *Nature* 422:716–719
- Pearson WH (1977) Chemoreception in the blue crab, *Callinectes sapidus*. *Biol Bull* 153:346–354
- Pickering AD, Pottinger TG, Carragher J, Sumpter JP (1987) The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. *Gen Comp Endocr* 68:249–259
- Pollock MS, Chivers DP, Mirza RS, Wisenden BD (2003) Fathead minnows, *Pimephales promelas*, learn to recognize chemical alarm cues of introduced brook stickleback, *Culaea inconstans*. *Environ Biol Fish* 66:313–319
- Pollock MS, Friesen RG, Pollock RJ, Kusch RC, Chivers DP (2005) The avoidance response of fathead minnows to chemical alarm cues: understanding the effects of donor gender and breeding condition. *Chemoecology* 15:205–209
- Pottinger TG (1992) The influence of social interaction on the acclimation of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to chronic stress. *J Fish Biol* 41:435–447
- Riley SC, Tatara CP, Scheurer JA (2005) Aggression and feeding of hatchery-reared and naturally reared steelhead (*Oncorhynchus mykiss*) fry in a laboratory flume and a comparison with observations in natural streams. *Can J Fish Aquat Sci* 62:1400–1409
- Scholz NL, Truelove NK, French BL, Berejikian BA, Quinn TP, Casillas E, Collier TK (2000) Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). *Can J Fish Aquat Sci* 57:1911–1918
- Scott AP, Ellis T (2007) Measurement of fish steroids in water—a review. *Gen Comp Endocr* 153:392–400
- Scott AP, Liley NR, Vermeirssen ELM (1994) Urine of reproductively mature female rainbow-trout, *Oncorhynchus-mykiss* (Walbaum), contains a priming pheromone which enhances plasma-levels of sex steroids and gonadotropin-II in males. *J Fish Biol* 44:131–147
- Scott AP, Sloman KA, Rouleau C, Wood CM (2003) Cadmium disrupts behavioral and physiological responses to alarm substances in juvenile rainbow trout (*Oncorhynchus mykiss*). *J Exp Biol* 206:1779–1790
- Sherman ML, Moore PA (2001) Chemical orientation of brown bullheads, *Ameiurus nebulosus*, under different flow conditions. *J Chem Ecol* 27:2301–2318
- Siefkes MJ, Winterstein SR, Li W (2005) Evidence that 3-keto petromyzonol sulphate specifically attracts ovulating female sea lamprey, *Petromyzon marinus*. *Anim Behav* 70:1037–1045
- Sloman KA, Armstrong JD (2002) Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena. *J Fish Biol* 61:1–23
- Smith RJF (1981) Effect of food deprivation on the reaction of Iowa darters (*Etheostoma exile*) to skin extract. *Can J Zool* 59:558–560
- Smith RJF (1997) Does one result trump all others? A response to Magurran, Irving and Henderson. *P Roy Soc B-Biol Sci* 264:445–450
- Sorensen PW (1992) Hormonally derived sex-pheromones in goldfish—a model for understanding the evolution of sex-pheromone systems in fish. *Biol Bull* 183:173–177
- Sorensen PW, Stacey NE (2004) Brief review of fish pheromones and discussion of their possible uses in the control of non-indigenous teleost fishes. *New Zeal J Mar Fresh* 38:399–417
- Sorensen PW, Vrieze LA (2003a) The chemical ecology and potential application of the sea lamprey migratory pheromone. *J Great Lakes Res* 29:66–84
- Sorensen PW, Vrieze LA (2003b) The chemical ecology and potential application of the sea lamprey migratory pheromone. *J Great Lakes Res* 29:66–84
- Sorensen PW, Stacey NE, Chamberlain KJ (1989) Differing behavioral and endocrinological effects of two female sex pheromones on male goldfish. *Horm Behav* 23:317–332
- Sorensen PW, Christensen TA, Stacey NE (1998) Discrimination of pheromonal cues in fish: emerging parallels with insects. *Curr Opin Neurobiol* 8:458–467
- Sorensen PW, Fine JM, Dvornikovs V, Jeffrey CS, Shao F, Wang J, Vrieze LA, Anderson KR, Hoye TR (2005) Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. *Nat Chem Biol* 1:324–328
- Spehr M, Kelliher KR, Li H-H, Boehm T, Leinders-Zufall T, Zufall F (2006) Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. *J Neurosci* 26:1961–1970
- Stacey N (2003) Hormones, pheromones and reproductive behavior. *Fish Physiol Biochem* 28:229–235
- Stacey N, Chojnacki A, Narayanan A, Cole T, Murphy C (2003) Hormonally derived sex pheromones in fish: exogenous cues and signals from gonad to brain. *Can J Physiol Pharm* 81:329–341
- Teeter J (1980) Pheromone communication in sea lampreys (*Petromyzon-marinus*)—implications for population management. *Can J Fish Aquat Sci* 37:2123–2132
- von Frisch K (1938) Zur Psychologie des Fischeschwarmes. *Naturwissenschaften* 26:601–606
- von Frisch K (1941) Über einen Schreckstoff der Fischhaut und seine biologische Bedeutung. *Z vergl Physiol* 29:46–145
- Vrieze LA, Sorensen PW (2001) Laboratory assessment of the role of a larval pheromone and natural stream odor in spawning stream localization by migratory sea lamprey (*Petromyzon marinus*). *Can J Fish Aquat Sci* 58:2374–2385
- Wagner CM, Jones ML, Twohey MB, Sorensen PW (2006) A field test verifies that pheromones can be useful for sea lamprey (*Petromyzon marinus*) control in the Great Lakes. *Can J Fish Aquat Sci* 63:475–479
- Wagner CM, Twohey MB, Fine JM (2009) Conspecific cueing in the sea lamprey: do reproductive migrations consistently follow the most intense larval odour? *Anim Behav* 78:593–599
- Weissburg MJ, Zimmer-Faust RK (1994) Odor plumes and how blue crabs use them in finding prey. *J Exp Biol* 197:349–375
- Wisenden BD (2008) Active space of chemical alarm cue in natural fish populations. *Behaviour* 145:391–407
- Wisenden BD, Chivers DP, Smith RJF (1994) Risk-sensitive habitat use by brook stickleback (*Culaea inconstans*) in areas associated with minnow alarm pheromone. *J Chem Ecol* 20:2975–2983
- Wisenden BD, Klitzke J, Nelson R, Friedl D, Jacobson PC (2004a) Predator-recognition training of hatchery-reared walleye (*Stizostedion vitreum*) and a field test of a training method using yellow perch (*Perca flavescens*). *Can J Fish Aquat Sci* 61:2144–2150
- Wisenden BD, Vollbrecht KA, Brown JL (2004b) Is there a fish alarm cue? Affirming evidence from a wild study. *Anim Behav* 67:59–67
- Wyatt TD (2003) Pheromones and animal behaviour: communication by smell and taste. Cambridge University Press, New York
- Yambe H, Yamazaki F (2001) A releaser pheromone that attracts methyltestosterone-treated immature fish in the urine of ovulated female rainbow trout. *Fisheries Sci* 67:214–220
- Yambe H, Munakata A, Kitamura S, Aida K, Fusetani N (2003) Methyltestosterone induces male sensitivity to both primer and releaser pheromones in the urine of ovulated female masu salmon. *Fish Physiol Biochem* 28:279–280
- Yambe H, Kitamura S, Kamio M, Yamada M, Matsunaga S, Fusetani N, Yamazaki F (2006) L-Kynurenine, an amino acid identified as a sex pheromone in the urine of ovulated female masu salmon. *P Natl Acad Sci USA* 103:15370–15374
- Yun S-S, Scott AP, Li W (2003) Pheromones of the male sea lamprey, *Petromyzon marinus* L.: structural studies on a new compound,

- 3-keto allocholic acid, and 3-keto petromyzonol sulfate. *Steroids* 68:297–304
- Zimmer RK, Commins JE, Browne KA (1999) Regulatory effects of environmental chemical signals on search behavior and foraging success. *Ecology* 80:1432–1446
- Zimmer-Faust RK, Finelli CM, Pentcheff ND, Wethey DS (1995) Odor plumes and animal navigation in turbulent water-flow—a field-study. *Biol Bull* 188:111–116