Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract: Neurotoxic pesticides are known to contaminate surface waters that provide habitat for salmonids, including some listed for protection under the U.S. Endangered Species Act. Despite their widespread use, the impacts of these pesticides on the neurological health of wild salmon are not well understood. Of particular concern are the organophosphate and carbamate insecticides that block synaptic transmission by inhibiting neuronal acetylcholinesterase. Here we assess the effects of diazinon, an organophosphate insecticide, on alarm pheromone induced antipredator responses and homing behavior in chinook salmon (*Oncorhynchus tshawytscha*). Nominal exposure concentrations (0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$) were chosen to emulate diazinon pulses in the natural environment. In the antipredator study, diazinon had no effect on swimming behavior or visually guided food capture. However, the pesticide significantly inhibited olfactory-mediated alarm responses at concentrations as low as 1.0 $\mu g \cdot L^{-1}$. Similarly, homing behavior was impaired at 10.0 $\mu g \cdot L^{-1}$. Our results suggest that olfactory-mediated behaviors are sensitive to anticholinesterase neurotoxicity in salmonids and that short-term, sublethal exposures to these insecticides may cause significant behavioral deficits. Such deficits may have negative consequences for survival and reproductive success in these fish.

Résumé : On sait que les pesticides neurotoxiques contaminent les eaux de surface qui constituent l'habitat des salmonidés, notamment de certains poissons désignés comme protégés par la loi américaine sur les espèces en danger («Endangered Species Act»). Malgré leur usage répandu, on ne connaît pas bien les impacts de ces pesticides sur la santé neurologique des saumons sauvages. On s'inquiète particulièrement des effets des insecticides à base d'organophosphate et de carbamate, qui bloquent le transfert synaptique en inhibant l'acétylcholinesterase (AchE) dans les neurones. Nous évaluons ici les effets du diazinon, un insecticide organophosphaté, sur les réactions antiprédateur induites par les phéromones d'alarme et sur le comportement de retour chez le saumon quinnat (*Oncorhynchus tshawytscha)*. Nous avons choisi des concentrations nominales d'exposition (0,1, 1,0 et 10,0 $\mu g \cdot L^{-1}$) pour simuler les administrations de diazinon dans le milieu naturel. Dans l'étude sur le comportement antiprédateur, le diazinon n'avait aucun effet sur le comportement de nage ni sur la capture de nourriture à vue. Toutefois, le pesticide inhibait de façon significative les réponses d'alarme médiées par l'olfaction à des concentrations de 1,0 $\mu g \cdot L^{-1}$. De même, le comportement de retour était troublé à 10,0 $\mu g \cdot L^{-1}$. Nos résultats permettent de penser que les comportements médiés par l'olfaction sont sensibles à la neurotoxicité anticholinesterase chez les salmonidés, et que des expositions sublétales de courte durée à ces insecticides peuvent causer d'importants déficits comportementaux, déficits qui peuvent avoir des conséquences négatives pour la survie et le succès de reproduction chez ces poissons.

[Traduit par la Rédaction]

Introduction

Wild Pacific salmon (*Oncorhynchus* sp.) populations are declining throughout the western United States (e.g., Myers et al. 1998). For example, several salmon stocks (defined as evolutionarily significant units) in the states of California, Oregon, Washington, and Idaho have recently been listed for protection under the U.S. Endangered Species Act (ESA). A significant factor in these declines is the deterioration or loss of freshwater habitat (National Research Council 1996). Land-use activities (e.g., forestry, agriculture, grazing, mining, and urban and industrial development) have complex

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and mostly negative consequences for salmon productivity. The effects of physical disturbances such as diking, erosion, channelization, sedimentation, loss of riparian vegetation, and loss of large woody debris on salmon productivity are reasonably well known (National Research Council 1996). However, many western river systems also receive substantial inputs of toxic chemicals, and the impacts of these compounds on salmon survival and reproductive health are poorly understood.

Rivers and streams in the western United States are routinely contaminated by a diverse mixture of insecticides, herbicides, and other biocidal compounds. Recent monitoring studies by the U.S. Geological Survey found 49 different pesticides in the surface waters of California's Central Valley (Dubrovsky et al. 1998), 50 in Oregon's Willamette basin (Wentz et al. 1998), and 23 in urban streams in the Puget Sound basin (U.S. Geological Survey 1999). Although these compounds are prevalent in streams and rivers that provide habitat for ESA-listed salmonids, the effects of individual pesticides and pesticide mixtures on salmon health are largely unknown. Consequently, there is considerable scientific uncertainty with respect to what role, if any, pesticides play in the decline of wild populations.

In general, the organophosphate and carbamate classes of insecticides are more acutely lethal to fish than other pesticides (EXTOXNET; pesticide information available at http://ace.orst.edu/info/extoxnet/). These compounds are neurotoxins that inhibit the activity of acetylcholinesterase (AChE), an enzyme that terminates acetylcholine-mediated, or cholinergic, neurotransmission. The enzyme is widely expressed throughout the fish nervous system (Weiss 1958; Ferenczy et al. 1997; Sturm et al. 1999). Correspondingly, AChE inhibition can lead to a distributed loss of nervous system function. At high concentrations, exposures to anticholinesterase neurotoxins produce spasms, paralysis, and eventual death (Post and Schroeder 1971). Organophosphates and carbamates frequently contaminate western rivers; for example, they account for 12 of 19 insecticides detected in the surface waters of California's Central Valley (Dubrovsky et al. 1998).

Diazinon, an organophosphate insecticide, is commonly detected in the aquatic environment (e.g., Dubrovsky et al. 1998). Diazinon is transported into rivers largely via stormwater runoff (Domagalski 1996), with rain events producing pesticide pulses in rivers and streams. These pulses are typically narrow and well defined, lasting from a few days to weeks (Kuivila and Foe 1995). While in-stream concentrations of diazinon have been measured as high as 36.8 $\mu g \cdot L^{-1}$ (Menconi and Cox 1994), they are more commonly less than 10.0 $\mu g \cdot L^{-1}$. A typical pulse of diazinon lasting for only a few days at concentrations of less than 10.0 $\mu g \cdot L^{-1}$ is unlikely to kill fish outright. The acute toxicity (96-h LC_{50}) of diazinon has not been determined for chinook salmon (*Oncorhynchus tshawytscha*), but the value is probably similar to the known LC_{50} values for congeneric rainbow trout (*Oncorhynchus mykiss*) (839 $\mu g \cdot L^{-1}$) or cutthroat trout (*Oncorhynchus clarki*) (2620 $\mu g \cdot L^{-1}$) (Novartis Crop Protection, Inc. 1997). If so, there is a substantial (approximately 2–3 orders of magnitude) difference between concentrations of diazinon commonly found in the environment and those required to produce acute lethality in the laboratory. Chronic toxicity (e.g., reduced egg production) has been reported in sheepshead minnow (*Cyprinodon variegatus*) at diazinon concentrations as low as 0.47 $\mu g \cdot L^{-1}$ (Goodman et al. 1979). However, chronic exposures can last for 1 or more months, which rarely, if ever, occurs under natural conditions. Thus, based on the results of conventional acute and chronic toxicity assays, it has been concluded that Pacific salmon are not at risk from the direct effects of diazinon in freshwater habitat (Novartis Crop Protection, Inc. 1997).

However, the conventional toxicity studies on which that conclusion was based were not explicitly designed to measure nervous system function. For example, conventional assays do not consider potential injury to the fish olfactory system despite the fact that sensory neurons are directly exposed to dissolved toxicants in the water. In fish, the detection of chemical cues (signal transduction) occurs within the olfactory rosettes, which are the principal structures in the fish olfactory organ. Olfactory receptor neurons, which bind odorant molecules, are embedded within a sensory epithelium that lines the folded lamellae of the rosettes (Zeiske et al. 1992). Chemosensory perception can be altered when dissolved neurotoxins either (*i*) compete with natural odorants for binding sites on membrane-associated receptor proteins, (*ii*) modulate the activation properties of these receptor proteins, or (*iii*) are translocated to the cytosol of the sensory neuron where they modify intracellular signalling events (Klaprat et al. 1992). The olfactory nervous system is sensitive to a wide range of dissolved contaminants (Sutterlin 1974; Brown et al. 1982; Klaprat et al. 1992), and a loss of olfactory capacity could interfere with many important behaviors in fish, including feeding, defense, schooling, migration, and reproduction (Kleerekoper 1969).

Recently, Moore and Waring (1996) found that environmentally relevant exposures to diazinon can desynchronize the reproductive physiology of prespawning Atlantic salmon (*Salmo salar*) by inhibiting the male's perception of a priming pheromone. Exposures to carbofuran, another anticholinesterase pesticide, resulted in a similar loss of function (Waring and Moore 1997). Sublethal exposures to carbofuran have also been shown to inhibit AChE enzymatic activity in the olfactory rosettes and the olfactory bulb of the common carp (*Cyprinus carpio*) (Haubruge and Toutant 1997). Collectively, these studies suggest that AChE plays an essential role in signal transduction within the olfactory epithelium. In addition, AChE inhibition may interfere with the normal integration of chemical cues within the olfactory forebrain.

Because chinook salmon rely on a wide range of olfactory-mediated behaviors for survival and reproductive success, a loss of olfactory capacity, while subtle, could have important negative consequences for the health of individual fish. In addition, olfactory cues provide the basis for imprinting and homing, which are critical for maintaining the integrity of wild populations (Dittman and Quinn 1996). Here, we use specific behavioral assays to determine whether diazinon disrupts olfactory nervous system function in chinook salmon. We show that sublethal, environmentally relevant exposures are sufficient to impair both alarm pheromone induced antipredator behavior and homing behavior in juvenile

and adult animals, respectively. These findings are discussed in the context of freshwater habitat quality and the management of ESA-listed species in western river systems.

Materials and methods

Antipredator study

Chemical alarm signal induced antipredator behaviors were measured using an experimental design modified from Berejikian et al. (1999). Eyed chinook salmon eggs were obtained from the Minter Creek Hatchery, Pierce County, Wash. The eggs were incubated and hatched in a model stream at the National Marine Fisheries Service Manchester Experimental Station (Manchester, Wash.). The fish were reared in a circular tank (1.2 m in diameter) on a 16 h light : 8 h dark cycle and fed a standard commercial salmon diet several times daily, 5 days per week. A stock conspecific skin extract (alarm stimulus) was prepared by homogenizing approximately 100 cm^2 of skin from 23 fish in 1 L of deionized water. The extract was filtered twice through a polyester filter floss, aliquoted, and stored at –20°C.

Behavorial trials were carried out indoors in ten 170-L aquaria positioned within two nearly identical flumes (9.0 m long by 1.5 m wide; see Berejikian et al. (1999) for a complete description of tank design). In brief, each aquarium was constructed of opaque glass on three sides. The tanks were lined with gravel, and uniform overhead lighting was provided by a solid bank of wide-spectrum fluorescent lights. A 15-cm^2 tile was situated approximately 5 cm above the gravel to provide overhead cover, and a 2.5-cm-high plastic ring was placed around the tile to add lateral cover. The water used during the maintenance, exposure, and testing of fish was gravity-fed from an on-site well. Water temperatures ranged between 10 and 14°C, and chemical properties were pH 8.0, total hardness (as $CaCO_3$) 65 $mg \cdot L^{-1}$, conductivity 150 $\mu mho \cdot cm^{-1}$, sodium 5.2 $mg \cdot L^{-1}$; magnesium 7.3 $mg \cdot L^{-1}$, and calcium 14 $mg \cdot L^{-1}$. Water was introduced at a rate of 6 $L \cdot min^{-1}$ via a polyvinyl tube that terminated middepth on one side of the aquarium, and depth was maintained at 25 cm by a double siphon on the opposite side of the tank.

It has been previously shown in Atlantic salmon parr that a brief exposure to diazinon (30 min at 1 $\mu g \cdot L^{-1}$) is sufficient to reduce the responsiveness of the olfactory epithelium to an odor stimulus. In addition, the olfactory epithelium does not recover within the first few hours postexposure (Moore and Waring 1996). Based on these findings, we chose to expose chinook salmon parr to diazinon for 2 h and allow them to recover for 1 h before initiating the antipredator behavioral trials. Diazinon exposures were conducted in 20-L opaque glass aquaria. Exposure concentrations (0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$) were prepared fresh each day by dissolving an analytical-grade stock of diazinon (Chem Services) in a small volume of acetone (0.01% final concentration). The diazinon–acetone mixtures were then diluted into 10 L of well water. Acetone alone (0.01%) was used as a vehicle control.

Diazinon exposures and behavioral testing were conducted in the following sequence. On the day prior to testing, a single juvenile chinook salmon was placed in each of the 170-L aquaria. The fish were allowed to acclimate for at least 24 h, during which they were fed a single meal (1.5% of body weight). On the day of the trial, individual fish were removed from their tanks and transferred to separate 20-L exposure aquaria that had been previously treated with diazinon or acetone as a vehicle control. After a 2-h exposure, the fish was returned to the 170-L test aquarium and allowed to reacclimate for 1 h. Subsequently, approximately 3 mL of live *Daphnia* (collected fresh from a local pond) was introduced into the tank via the inflow tube. After a 2-min interval, prestimulus feeding behavior was recorded for 8 min. The conspecific skin extract (100 μL) was then added through the water inflow tube. After a 10-s delay, antipredator behaviors were recorded during an 8-min poststimulus interval.

Prestimulus feeding behaviors and the poststimulus antipredator response were recorded using behavioral data acquisition software (Observer 2.0). Specifically, we measured the frequency of food strikes, the amount of time spent motionless, and the amount of time spent in the lower, middle, and upper third of the water column. Fish were defined as motionless if they remained stationary (moved less than 1 cm) for 3 s. During the alarm response, many animals maintained a stationary position in the water column by rapidly fanning their pectoral fins. The frequency of darting behavior (burst swimming) and amount of time spent under cover were also measured; however, as reported previously (Berejikian et al. 1999), these behaviors were infrequent and we did not consider them significant antipredator behaviors.

All trials were conducted blind, i.e., the observer had no knowledge of the treatment conditions. Overall, 17–20 individual juvenile chinook salmon were tested at each exposure concentration for a total of 75 fish (fork lengths between 4.7 and 6.3 cm, mean = 5.5 cm). Each fish was used only once, and the relationship between diazinon treatment and test aquarium was randomized between trials. In preliminary experiments, three control fish were exposed to a higher concentration of skin extract. The poststimulus behaviors of these animals were not included in the analysis.

Homing study

Homing behavior has previously been used to measure olfactory capacity in chinook salmon (Quinn et al. 1988). In the present study, a total of 160 maturing chinook salmon were collected from the University of Washington hatchery between October 9 and November 10, 1998. The pond was seined twice weekly, and returning males (age 1) were captured and transferred to a concrete raceway. The fish (fork lengths between 14.8 and 30.1 cm, mean = 23.7 cm) were in good condition, without lacerations or obvious signs of disease.

After each day of seining, the fish captured were immediately divided into four treatment groups and fin-clipped for identification. The following day, static 24-h exposures to diazinon (0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$ nominal concentrations) or a vehicle control (acetone, final concentration <0.01%) were conducted in four 800-L tanks filled with lake water. Diazinon dilutions were prepared fresh each day from an analytical-grade stock solution (Chem Services). Each tank was fitted with a Living Stream® chiller to maintain aeration and a constant water temperature (13–14°C).

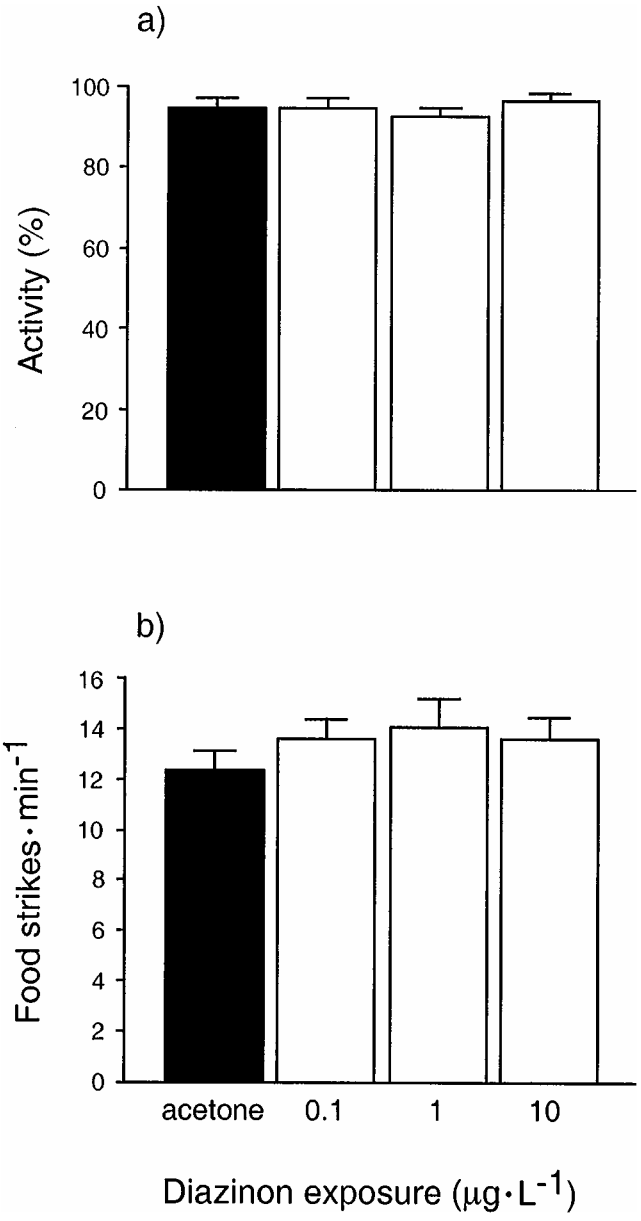
Following exposures, fish were transferred to a 775-L stainless steel tank and transported to a freshwater release site approximately 2 km downstream from the hatchery. A total of 160 fish (40 per treatment group) were treated and released during the course of the experiment. Homing fish (i.e., those returning to the hatchery) were identified during subsequent seining operations at the hatchery that lasted until November 23. In addition, a total of 20 fish (five per treatment group) were placed together in a concrete raceway at the hatchery. These animals were in the same size range as the fish that were treated and released. They were monitored throughout the study to ensure that the diazinon exposures did not grossly reduce the longevity of animals used in the homing experiment.

Results

Antipredator study

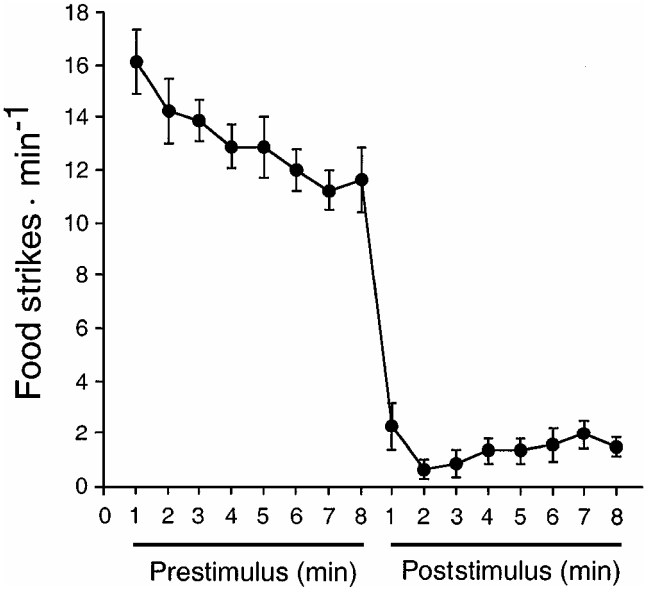
A 2-h diazinon exposure at nominal concentrations of 0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$ had no significant effect on either basal foraging behavior or swimming activity (Fig. 1). In general, individual fish fed actively throughout the 8-min prestimulus

Fig. 1. Short-term diazinon exposures have no effect on (*a*) swimming activity or (*b*) feeding behavior in chinook salmon parr. Individual fish were removed from their aquaria and exposed to diazinon (0.1, 1.0, and 10.0 μg·L^{-1} nominal concentrations) or a vehicle control (acetone) for 2 h. The fish were subsequently returned to their aquaria and allowed to reacclimate for 1 h. Food (live *Daphnia*) was added to the tank and the behavior of each fish was recorded (see Materials and methods) for 8 min. Values represent the mean ± SEM for 20 animals. Each animal was tested only once. There were no significant differences among treatment groups ($p > 0.05$, Fisher's test).



interval (Fig. 2). As previously reported (Berejikian et al. 1999), juvenile chinook salmon show stereotypic antipredator behaviors in response to a chemical alarm signal. Qualitatively, animals moved towards the bottom of the tank and became motionless when a conspecific skin extract was added to the tank. In addition, control fish abruptly stopped feeding in response to the skin extract (Fig. 2). The alarm

Fig. 2. Conspecific skin extract elicits an abrupt reduction in feeding behavior in control chinook salmon. Feeding activity was recorded for 8 min before (prestimulus) and after (poststimulus) the addition of the skin extract to the aquaria. Values are data from 1-min intervals and represent the mean ± SEM for 17 animals.



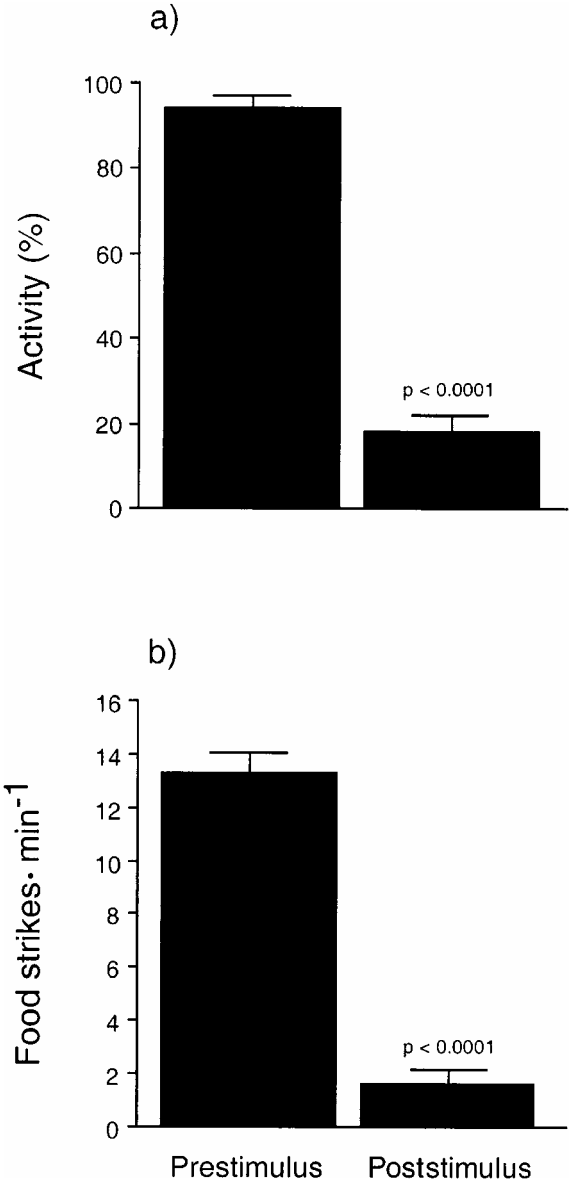
signal elicited a significant decrease in both swimming activity ($p < 0.001$, Fisher's test; Fig. 3*a*) and feeding behavior ($p < 0.001$, Fisher's test; Fig. 3*b*) in controls. The fish also tended to move to the lower third of the water column. However, several fish were already foraging in the lower part of the water column when the extract was introduced, so the pre- and post-stimulus differences in this measure were less pronounced.

Compared with control animals, diazinon-treated fish remained more active and fed more frequently when exposed to the conspecific skin extract (Fig. 4). Although some treated fish exhibited antipredator behaviors, many continued to forage in the presence of the alarm signal. The effect of diazinon on swimming and feeding behavior was significant at concentrations of 1 and 10.0 μg·L^{-1} ($p = 0.05$, Fisher's test), with the exception of the 10.0 μg·L^{-1} dose, which had a marginal effect ($p = 0.06$) on poststimulus swimming activity.

Homing study

Fewer diazinon-treated chinook salmon returned to the hatchery compared with control fish (Fig. 5). Overall returns were 40% (16 of 40) for control fish, 30% (12 of 40) for the 0.1 and 1.0 μg·L^{-1} exposure groups, and 15% (six of 40) for the 10.0 μg·L^{-1} exposure group. The effect of diazinon on homing success was significant at the 10.0 μg·L^{-1} exposure ($p < 0.01$, chi-square contingency analysis). Diazinon did not appear to have any gross effects on chinook salmon longevity. Compared with controls, there were no significant differences in the survival of diazinon-treated fish ($n = 5$ for each treatment group) that were held in raceways at the hatchery. Survival durations (mean ± SEM) for the different treatment groups were 26 ± 9 days for controls, 31 ± 11 days at 0.1 μg·L^{-1}, 24 ± 13 days at 1.0 μg·L^{-1}, and 25 ± 12 days at 10.0 μg·L^{-1}.

Fig. 3. Control chinook salmon significantly decrease both (*a*) swimming activity and (*b*) food capture behavior in response to the alarm stimulus ($p < 0.001$, Fisher's test). Values represent the mean ± SEM for 17 animals, and the data from an entire interval (prestimulus or poststimulus) are combined.

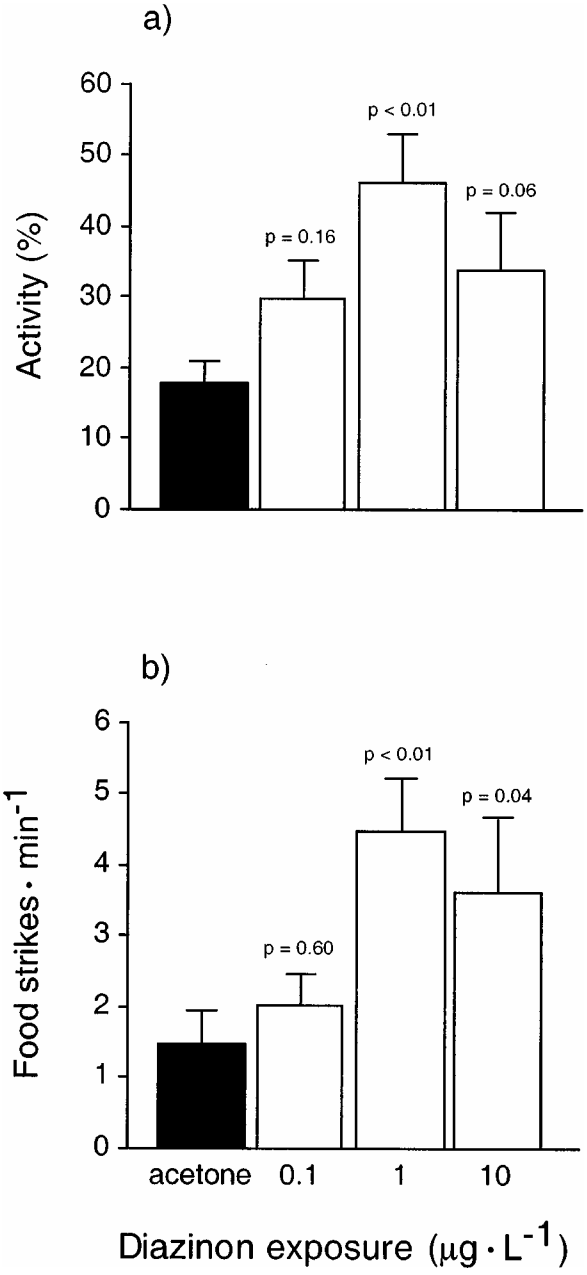


We were unable to test the hypothesis that diazinon-treated fish take longer to return to the University of Washington hatchery than controls. Returning fish were collected two or three times each week as part of normal hatchery operations. Consequently, we did not obtain precise temporal data on when fish from different treatment groups reentered the pond. Moreover, the small males used in this study occasionally avoided the net during seining. However, we found no significant differences in time to recapture (mean ± SEM) between control fish (11.8 ± 2.2 days) and animals exposed to diazinon at 0.1 $\mu g \cdot L^{-1}$ (11.0 ± 2.8 days), 1.0 $\mu g \cdot L^{-1}$ (11 ± 2.1 days), and 10.0 $\mu g \cdot L^{-1}$ (7.5 ± 3.0 days).

Discussion

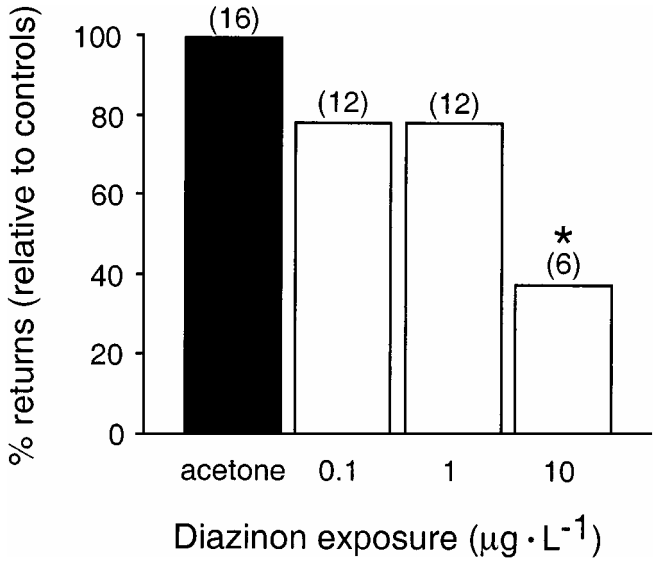
Olfaction plays an important role in the complex life his-

Fig. 4. Antipredator behaviors are reduced in diazinon-exposed chinook salmon. Control fish responded to the conspecific skin extract by reducing their foraging activity and freezing, i.e., poststimulus fish were (*a*) largely inactive and (*b*) fed only infrequently (solid bar in Figs. 4*a* and 4*b*, respectively). The magnitude of the antipredator response was reduced in diazinon-exposed fish (2 h at 0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$), and they were (*a*) more active and (*b*) fed more often than controls. The effect of diazinon was significant at the 1.0 and 10.0 $\mu g \cdot L^{-1}$ exposures ($p = 0.05$, Fisher's test) with the exception of the marginal ($p = 0.06$) effect of 10.0 $\mu g \cdot L^{-1}$ on poststimulus swimming activity.



tories of Pacific salmon. We found that environmentally realistic exposures to the insecticide diazinon significantly disrupt olfactory-mediated behavior in chinook salmon. Our findings, together with recent studies of Atlantic salmon (Moore and Waring 1996, 1998; Waring and Moore 1997), demonstrate that the salmon olfactory nervous system is par-

Fig. 5. A 24-h exposure to diazinon disrupts homing in chinook salmon males. Compared with controls, fewer diazinon-exposed fish returned to the University of Washington hatchery from a release site approximately 2 km downstream. A total of 40 fish were released in each treatment group. Numbers in parentheses indicate the total number of fish returning. The effect of diazinon was significant at the 10.0 $\mu g \cdot L^{-1}$ exposure ($p < 0.01$, chi-square contingency analysis).



ticularly sensitive to dissolved neurotoxins. Diazinon has now been shown, in salmonids, to impair reproductive priming (Moore and Waring 1996) as well as predator avoidance and homing behaviors (present study). The survival and reproductive success of wild Pacific salmon may, therefore, be compromised in river systems that are periodically contaminated with diazinon and other neurotoxic pesticides.

Antipredator behavior

Overall, the results of the antipredator experiment are consistent with the hypothesis that diazinon disrupts olfactory nervous system function in juvenile fish. Our findings confirm earlier studies (Brown and Smith 1997, 1998; Berejikian et al. 1999) demonstrating a chemical alarm system in salmonids. Moreover, when compared with controls, diazinon-exposed chinook salmon parr showed a reduced antipredator response to the conspecific skin extract (they were more active and fed more often in the presence of the alarm stimulus). Alarm behaviors reduce the likelihood that an animal will be preyed upon during an encounter with a predator (Smith 1992). For example, Mathis and Smith (1993) found that fathead minnows (*Pimephales promelas*) exposed to a conspecific alarm pheromone survived predation by a northern pike (*Esox lucius*) for approximately 40% longer than fathead minnows that were not exposed to the pheromone. Although not tested directly, the disruption of the alarm response by diazinon would likely increase the vulnerability of chinook salmon parr to predation.

In general, environmental toxins can reduce the ecological performance of a prey animal by adversely affecting its behavior (Mesa et al. 1994). In chinook salmon parr, we have shown that diazinon interferes with the early olfactory detection of a nearby predator, which is critical for escape and survival (Mesa et al. 1994). As a consequence, diazinon-exposed parr would be in a substandard condition at the time of a predatory interaction. For example, the alarm substance elicited subtle shifts in behavior during the poststimulus observation period. Individual fish would sometimes continue to search for food, but in a more restricted area, or they would continue to strike at food, but only if a *Daphnia* drifted near their stationary position. Presumably, the decision to forage in the presence of an alarm signal reflects a balance between each animal's appetitive or motivational state and its individual perception of predation risk. Decision-making, whereby fish balance the trade-off between food acquisition and the risk of mortality, is an important factor in predator–prey interactions (Mesa et al. 1994). For example, juvenile coho salmon (*Oncorhynchus kisutch*) restrict their foraging behavior following the visual detection of a predator (Dill and Fraser 1984; Martel and Dill 1993). In the present study, control fish sharply reduced their feeding activity in response to the alarm signal. By contrast, some diazinon-treated fish continued to forage at levels that were inappropriate for the situation, perhaps because they were unable to accurately gauge the potential of a predatory encounter.

The results from the antipredator study suggest that the olfactory nervous system is more sensitive to anticholinesterase neurotoxins when compared with the motor nervous system (e.g., the networks that underlie swimming behavior). Diazinon had no observed effect on swimming activity or visually guided food capture behavior at concentrations that significantly disrupted alarm signal evoked antipredator behaviors. Swimming performance is a common behavioral measure of sublethal anticholinesterase toxicity in salmonids and other fishes (e.g., Cripe et al. 1984; Little et al. 1990; Van Dolah et al. 1997). Although previous studies have reported anticholinesterase-induced swimming impairment, the thresholds for behavioral dysfunction were generally at concentrations or durations that exceed conditions in the natural environment. By these measures, it could be interpreted that anticholinesterases pose little risk for wild salmon. However, our results suggest that motor networks may be relatively insensitive to anticholinesterases. Consequently, toxicity thresholds derived from swimming assays may underestimate the susceptibility of the nervous system as a whole.

Homing

In the homing study, fewer diazinon-treated fish returned to the University of Washington hatchery compared with controls. Chinook salmon rely on olfactory cues to guide their upstream movements during the freshwater phase of their homeward migration (Hasler and Scholz 1983), and the weight of evidence suggests that diazinon-exposed fish were less able to smell and thus were less able to navigate back to their natal stream (Moore and Waring 1996). However, the results of the homing study should be viewed as preliminary for several reasons. First, the overall return of maturing males to the hatchery in the fall of 1998 was low, which limited the number of fish in each treatment group. Second, posttreatment returns were small in all four exposure groups. Only 40% of control fish returned to the hatchery, compared with approximately 80% in previous studies of homing in older salmon (e.g., Quinn et al. 1988). The reason for this

discrepancy is not clear. It may reflect differences in the ages of the fish used or environmental conditions or both. Third, the hatchery pond was seined biweekly, and without precise temporal return data, we were unable to determine whether diazinon-treated fish took longer to home relative to controls. Finally, although our longevity experiment indicates that it is unlikely that diazinon exposures caused a delayed gross mortality, we cannot rule out the possibility that exposures may have subtly diminished postrelease survival. This could also account for the observed differences in returns. Nevertheless, significantly fewer fish returned to their natal stream to spawn after a short-term exposure to diazinon.

Dissolved neurotoxins may increase the incidence of straying among wild and hatchery-reared salmon that must traverse pesticide-contaminated rivers during their homeward migration. Straying, in which fish return to nonnatal streams to spawn, is an adaptive mechanism for colonizing new habitat and avoiding unfavorable local conditions (reviewed by Quinn 1993). However, straying of hatchery fish could compromise the genetic integrity of wild populations (Quinn 1993). Reciprocally, straying of wild salmon can diminish the number of animals remaining to spawn in a given stream. A loss of olfactory nervous system function would presumably lead to an increase in straying, since anosmic (nares-occluded) fish are unable to home (Wisby and Hasler 1954). Since sublethal exposures to pesticides and other contaminants can render fish functionally anosmic (reviewed by Klaprat et al. 1992), the presence of these toxicants in the freshwater environment could increase straying, thereby undermining the genetic integrity of ESA-listed species. This represents an important area for future research, especially since the factors that determine normal patterns of straying in wild and hatchery salmon are still poorly understood (Quinn 1993).

Management implications

Conventional chronic and acute toxicity studies, and the LC_{50} experimental paradigm in particular, may underestimate thresholds for neurobehavioral toxicity in salmonids. Consequently, the use of mortality data may not be appropriate for risk assessment in the context of the ESA. For example, our data and the study by Moore and Waring (1996) indicate that environmentally relevant exposures to diazinon can disrupt olfactory capacity in the context of survival and reproductive success, both of which are key management considerations under the ESA. These findings contrast with an ecological risk assessment of diazinon in the Sacramento and San Joaquin River basins (Novartis Crop Protection, Inc. 1997), which concluded that salmon are unlikely to be at risk from direct acute effects of diazinon residues in the water. Importantly, the latter study relied extensively on mortality (LC_{50}) measurements, an approach that has several drawbacks in the context of the ESA. First, to our knowledge, there have been no reports of diazinon-induced fish kills in western streams. Given this, acute mortality has little or no relevance to conditions in the natural environment. Second, LC_{50} studies are not explicitly designed to measure nervous system function. Consequently, it is impossible to accurately predict from LC_{50} values the exposures at which diazinon could cause sublethal behavioral disorders in wild salmon. Third, the LC_{50} values for diazinon in representative salmonids are approximately 2–3 orders of magnitude higher than the highest concentrations of pesticide that are commonly detected in the aquatic environment. This represents a perceived margin of safety that, when taken alone, may significantly underestimate the actual risk that ambient levels of diazinon and related pesticides pose for salmon.

Diazinon pulses are common in western rivers and streams. This insecticide was detected, at concentrations greater than 0.01 $\mu g \cdot L^{-1}$, in 71% of the surface water samples from California's San Joaquin and Tulare basins (Dubrovsky et al. 1998) and in 35% of the samples taken from Oregon's Willamette basin (Wentz et al. 1998). Similarly, diazinon was recently found at 100% of the sites sampled in an analysis of urban streams in Puget Sound, Washington State (U.S. Geological Survey 1999). Although in-stream pulses of diazinon alone may reach levels high enough to cause behavioral dysfunction in wild salmon, diazinon is only one of many current-use pesticides that cooccur in the aquatic environment. Organophosphates and carbamates both inhibit AChE (Millard and Broomfield 1995), and they are likely to have an additive impact on AChE-mediated functions in the fish nervous system. In the surface waters of California's Central Valley, the organophosphates azinphos-methyl, chlorpyrifos, diazinon, disulfoton, fonofos, malathion, methyl parathion, and terbufos and the carbamates aldicarb, carbaryl, carbofuran, and methomyl all cooccur (Dubrovsky et al. 1998). The additive effects of these pesticides on the salmon nervous system have not been studied, and it remains an important area for future research.

In conclusion, a properly functioning nervous system is critical for the expression of animal behavior. We have shown that environmentally relevant exposures to diazinon are sufficient to disrupt olfactory-mediated behaviors in chinook salmon. These behavioral deficits may, in turn, have negative consequences for the survival and reproductive success of animals under natural conditions. Since diazinon and other neurotoxic pesticides with a similar mode of action are known to contaminate river systems in the western United States that provide freshwater habitat for several ESA-listed species, they represent an unknown but potentially significant obstacle to salmon recovery efforts.

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References

Berejikian, B.A., Smith, R.J.F, Tezak, E.P., Schroder, S.L., and Knudsen, C.M. 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. Can. J. Fish. Aquat. Sci. **56**: 830–838.

Brown, G.E., and Smith, R.J.F. 1997. Conspecific skin extracts elicit antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Zool. **75**: 1916–1922.

Brown, G.E., and Smith, R.J.F. 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, S.B., Evans, R.E., Thompson, B.E., and Hara, T.J. 1982. Chemoreception and aquatic pollutants. *In* Chemoreception in fishes. *Edited by* T.J. Hara. Elsevier, Amsterdam, The Netherlands. pp. 363–393.

Cripe, G.M., Goodman, L.R., and Hansen, D.J. 1984. Effect of chronic exposure to EPN and to Guthion on the critical swimming speed and brain acetylcholinesterase activity of *Cyprinidon variegatus*. Aquat. Toxicol. **5**: 255–266.

Dill, L.M., and Fraser, H.G. 1984. Risk of predation and the feeding behavior of juvenile coho salmon (*Oncorhynchus kisutch*). Behav. Ecol. Sociobiol. **16**: 65–71.

Dittman, A.H., and Quinn, T.P. 1996. Homing in Pacific salmon: mechanisms and ecological basis. J. Exp. Biol. **199**: 83–91.

Domagalski, J. 1996. Pesticides and pesticide degradation products in stormwater runoff: Sacramento River basin, California. Water Resour. Bull. **32**: 953–964.

Dubrovsky, N.M., Kratzer, C.R., Brown, L.R., Gronberg, J.M., and Burow, K.R. 1998. Water quality in the San Joaquin – Tulare basins, California, 1992–1995. U.S. Geol. Surv. Circ. 1159.

Ferenczy, J., Szegletes, T., Bálint, T., Abrahám, M., and Nemcsók, J. 1997. Characterization of acetylcholinesterase and its molecular forms in organs of five freshwater teleosts. Fish Physiol. Biochem. **16**: 515–529.

Goodman, L.R., Hansen, D.J., Coppage, D.L., Moore, J.C., and Matthews, E. 1979. Diazinon: chronic toxicity to, and brain acetylcholinesterase inhibition in, the sheepshead minnow, *Cyprinodon variegatus*. Trans. Am. Fish. Soc. **108**: 479–488.

Hasler, A.D., and Scholz, A.T. 1983. Olfactory imprinting and homing in salmon. Springer-Verlag, Berlin.

Haubruge, E., and Toutant, J.-P. 1997. L'acétylcholinésterase du système olfactif de la carpe *Cyprinus carpio* L. (Poissons, Cyprinidae): formes moléculaires et inhibition *in vivo* et *in vitro* par le carbofuran. Belg. J. Zool. **127**: 63–73.

Klaprat, D.A., Evans, R.E., and Hara, T.J. 1992. Environmental contaminants and chemoreception in fishes. *In* Fish chemoreception. *Edited by* T.J. Hara. Chapman and Hall, New York. pp. 321–341.

Kleerekoper, H. 1969. Olfaction in fishes. Indiana University Press, Bloomington, Ind.

Kuivila, K.M., and Foe, C.G. 1995. Concentrations, transport and biological effects of dormant spray pesticides in the San Francisco estuary, California. Environ. Toxicol. Chem. **14**: 1141–1150.

Little, E.E., Archeski, R.D., Flerov, B.A., and Kozlovskaya, V.I. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. Arch. Environ. Toxicol. **19**: 380–385.

Martel, G., and Dill, L.M. 1993. Feeding and agressive behaviors in juvenile coho salmon (*Oncorhynchus kisutch*) under chemically-mediated risk of predation. Behav. Ecol. Sociobiol. **32**: 365–370.

Mathis, A., and Smith, R.J.F. 1993. Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). Behav. Ecol. **4**: 260–265.

Menconi, M., and Cox, C. 1994. Hazard assessment of the insecticide diazinon to aquatic organisms in the Sacramento – San Joaquin river system. Admin. Rep. 94-2, California Department of Fish and Game, Rancho Cordova, Calif.

Mesa, M.G., Poe, T.P., Gadomski, D.M., and Peterson, J.H. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. J. Fish Biol. **45**: 81–96.

Millard, C.B., and Broomfield, C.A. 1995. Anticholinesterases: medical applications of neurochemical principles. J. Neurochem. **64**: 1910–1918.

Moore, A., and Waring, C.P. 1996. Sublethal effects of the pesticide Diazinon on olfactory function in mature male Atlantic salmon parr. J. Fish Biol. **48**: 758–775.

Moore, A., and Waring, C.P. 1998. Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Pest. Biochem. Physiol. **62**: 41–50.

Myers, J.M., Kope, R.G., Bryant, G.J., Teel, D., Lierheimer, L.J., Wainwright, T.C., Grant, W.S., Waknitz, F.W., Neely, K., Lindley, S.T., and Waples, R.S. 1998. Status review of chinook salmon from Washington, Idaho, Oregon, and California. U.S. Dep. Commer. NOAA Tech. Memo. NMFS-NWFSC-35.

National Research Council. 1996. Upstream: salmon and society in the Pacific Northwest. National Academy Press, Washington, D.C.

Novartis Crop Protection, Inc. 1997. An ecological risk assessment of diazinon in the Sacramento and San Joaquin river basins. Tech. Rep. 11/97, Novartis Crop Protection, Inc., Greensboro, N.C.

Post, G., and Schroeder, T.R. 1971. The toxicity of four insecticides to four salmonid species. Bull. Environ. Contam. Toxicol. **6**: 144–155.

Quinn, T.P. 1993. A review of homing and straying of wild and hatchery-produced salmon. Fish. Res. **18**: 29–44.

Quinn, T.P., Olson, A.F., and Konecki, J.T. 1988. Effects of anaesthesia on the chemosensory behavior of Pacific salmon. J. Fish Biol. **33**: 637–641.

Smith, R.J.F. 1992. Alarm signals in fishes. Rev. Fish Biol. Fish. **2**: 33–63.

Sturm, A., da Silva de Assis, H.C., and Hansen, P.-D. 1999. Cholinesterases of marine teleost fish: enzymological characterization and potential use in the monitoring of neurotoxic contamination. Mar. Environ. Res. **47**: 389–398.

Sutterlin, A.M. 1974. Pollutants and the chemical senses of aquatic animals — perspective and review. Chem. Senses Flavor, **1**: 167–178.

U.S. Geological Survey. 1999. Pesticides detected in urban streams during rainstorms and relations to retail sales of pesticides in King County, Washington. U.S. Geol. Surv. Fact Sheet 097-99.

Van Dolah, R.F., Maier, P.P., Fulton, M.H., and Scott, G.I. 1997. Comparison of azinphosmethyl toxicity to juvenile red drum (*Sciaenops ocellatus*) and the mummichog (*Fundulus heteroclitus*). Environ. Toxicol. Chem. **16**: 1488–1493.

Waring, C.P., and Moore, A.P. 1997. Sublethal effects of a carbamate pesticide on pheromonal mediated endocrine function in mature male Atlantic salmon (*Salmo salar*) parr. Fish Physiol. Biochem. **17**: 203–211.

Weiss, C.M. 1958. Determination of cholinesterase in the brain tissue of three species of fresh water fish and its inactivation in vivo. Ecology, **39**: 194–199.

Wentz, D.A., Bonn, B.A., Carpenter, K.D., Hinkle, S.R., Janet, M.L., Rinella, F.A., Uhrich, M.A., Waite, I.R., Laenen, A., and Bencala, K. 1998. Water quality in the Willamette Basin, Oregon, 1991–95. U.S. Geol. Surv. Circ. 1161.

Wisby, W.J., and Hasler, A.D. 1954. Effect of occlusion on migrating silver salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. **11**: 472–478.

Zeiske, E., Theisen, B., and Breuker, H. 1992. Structure, development, and evolutionary aspects of the peripheral olfactory system. *In* Fish chemoreception. *Edited by* T.J. Hara. Chapman and Hall, New York. pp. 13–39.