Re-evaluating eradication of nuisance species: invasion of the tunicate, *Ciona intestinalis*

Paul K Edwards and Brian Leung

*Front Ecol Environ* 2009; 7, doi:10.1890/070218

This article is citable (as shown above) and is released from embargo once it is posted to the *Frontiers* e-View site (www.frontiersinecology.org).

Please note: This article was downloaded from *Frontiers* e-View, a service that publishes fully edited and formatted manuscripts before they appear in print in *Frontiers in Ecology and the Environment*. Readers are strongly advised to check the final print version in case any changes have been made.
Many non-native tunicate species have recently invaded the coastal waters of North America and pose serious threats to marine ecosystems (McKindsey et al. 2007). The tunicates occur in such massive numbers that the substratum itself may be entirely obscured (Figure 1). In particular, an invasion by the tunicate Ciona intestinalis (henceforth “Ciona”) is considered to be at a “crisis level”, and is a major marine invasive species issue for the Department of Fisheries and Oceans Canada (DFO; T Landry pers comm). This species has a remarkably high fecundity and reproductive rate (Gray and Christie 1983), with several generations often present in a single season (Svane and Young 1989). Ciona is thought to have been introduced to the province of Prince Edward Island (PEI), Canada, by the bivalve aquaculture trade (Lambert and Lambert 1998). It is a “broadcast spawner”, freely releasing gametes into the water column; however, its transfer between bays seems relatively low (about one new bay per year), compared to the massive growth and spread within bays. Around PEI, Ciona has spread from first detection in 2004 to smothering densities in several bays in 2008. Ciona blankets aquaculture crops, putting 77% of Canada’s mussel farms in danger (DFO 2006). Mussel farmers who have spent decades developing their farms are losing their livelihoods (T Landry pers comm).

Despite its social relevance, current managerial guidance is insufficient to control Ciona. This may be because information on population biology rarely exists early in an invasion, and this has been used to justify inaction (Simberloff 2003). As a result, an invader will often have major impacts before action is taken (Mack et al. 2000). To minimize such impacts, managers need to speed up their rate of response, despite uncertainty and limited information (ie the precautionary principle; Kriebel et al. 2001). This may be possible, as only a subset of data is typically required to assess the feasibility of a given management option (Roe 1998; Simberloff 2003). Here, we identify the information required to assess the possibility for eradication of invasive species.

Eradication is an important concept in the management of biological invasions, but it is rarely considered in practice. This may be because managers commonly work with incomplete data and little or no practical guidance. Past eradication frameworks provide some useful criteria, but do not provide quantitative guidelines. Here, we argue that eradication is not always adequately considered, and we develop a framework for rapid assessment of its feasibility, despite limited data. This quantitative model offers criteria to rapidly assess the potential for eradication and provide estimates of the necessary effort and timing, and of the size of the target area. This framework is applied to a recent tunicate (Ciona intestinalis) invasion around Prince Edward Island, Canada, which is causing considerable economic damage to harvesters of blue mussels (Mytilus edulis). Our framework suggests that eradication may be feasible and, based on a cost–benefit analysis, could require only a > 16% chance of success to constitute a worthwhile risk.

In a nutshell:
- Eradication of non-native species is often dismissed prematurely, due to limited data and insufficient guidance for resource managers
- A quantitative, general framework for eradication has been developed to guide management of invaders with a minimum of population data
- This framework has been applied to a case study involving tunicates, and shows that eradication may be both feasible and economically desirable relative to current control efforts

Front Ecol Environ 2009; 7, doi:10.1890/070218

Department of Biology, McGill University, Montreal, QC, Canada *(paul.edwards1@mail.mcgill.ca)
Eradication of nuisance species

PK Edwards and B Leung

astonishing population growth rates and spread. We disagree, and suggest that it may be feasible to eradicate Ciona and other established invaders, and that this should be assessed explicitly.

We argue that eradication has been prematurely dismissed in practice, due to the lack of practical, quantitative steps for analysis. The few papers that have examined this important issue have provided basic criteria regarding eradication success (eg it is early in an invasion [Simberloff 2003]; the species has biological characteristics susceptible to control [Myers et al. 2000]; rates of removal are greater than rates of reproduction [Bomford and O’Brien 1995]). While these criteria are useful, and the Ciona invasion meets many of their requirements, they provide little instruction on the more practical elements of eradication that are of most interest to managers. These include cost, scope, and time and research required. Such explicit frameworks need to be developed to fully evaluate the potential for eradication of invasive species and to provide managers with these useful parameters. Here, we develop this general, quantitative framework for eradication, and identify the few key biological parameters that are required. Finally, we apply our framework to a case study involving Ciona.

Model

In formulating a conceptual model, we reduce complexity to five self-evident, general, core statements: (1) simply put, invaders get to new places and grow; (2) locations can become (re)infested from other invaded locations, limited by dispersal; (3) growth of populations follows life cycles, from larval or egg stage, to non-reproductive juveniles, to reproductive adults, which in turn produce more larvae (Figure 2); (4) if we can disrupt the system, we can stop reproducing adults from spawning, and the population will eventually go extinct; and (5) some stages may be more susceptible to management actions than others (Buhle et al. 2005). Our analysis of eradication follows from these statements.

To disrupt the cycle, we define a vulnerability “time window” (t_w) as a minimum range of susceptible, pre-reproductive ages of individuals (eg growth to a visible size or immobility below a certain age; Figure 2). Of course, reproductive individuals should be removed if possible. However, the crucial factor is that a subset of pre-reproductive stages can be eradicated, since this will eventually eliminate reproductive stages.

Next, we determine the number of passes (repeated treatments of a site) needed for eradication, using the time window and the progress along life cycles (Figure 2). We conceptualize the process as a “ratchet effect”. As younger juveniles mature, they enter the vulnerability time window. Each time a pass is made, all individuals within the window are removed. Thereafter, the life cycle ratchets up – younger individuals missed by the previous pass mature and become vulnerable, and are removed in the subsequent pass. Since the time window starts before the age of maturity, and since individuals are kept from aging beyond the window, no new adults develop. Thus, as a result of multiple passes on just one vulnerable stage, potential sources of propagules can be removed from a population, while existing adults are either removed directly or eventually experience natural mortality. The feasibility of this approach is increased because not all individuals need be eradicated at once – individuals outside the window may be unaffected.

Using knowledge of t_w, which defines both the length of time that juveniles are vulnerable and the maximum time within which a pass must be made, we can define an important criterion for eradication success. Assuming that spread can occur among local populations, cleared areas can become re-infested by other invaded areas within the bay. Therefore, a manager must be able to finish treating relevant local populations before missed juveniles mature beyond the window, become reproductive, and act as a continuing source for the system:

$$t_w > N t_1 + t_r$$

The total time for a pass is thus the treatment time of each population, t_r, multiplied by the number of local populations, N, plus an additional t_r, since time must be allotted to begin a second pass to treat the first population again. This must be done before missed juveniles in
the population mature beyond the window.

If treatment over the whole system is not logistically feasible, eradication may still be possible by sweeping over the system, applying overlapping treatments to a smaller area, which we term the “treatment zone”. Exchange of larvae among the populations within the treatment zone will be halted after a sufficient number of passes. However, populations outside of the treatment zone will be freely growing and spreading. If the treatment zone is larger than the maximum spread distance within the time window, some populations within the treatment zone would be out of the range of larval influx from outside populations (Figure 3a: B–D). The next treatment zone (Figure 3b) would overlap the re-infested populations (Figure 3a, b: E,F), but some local populations would remain free of infestation. This introduces the second eradication criterion: the size of the treatment zone must exceed spread within the time window.

The three components developed above – number of passes, time window, and treatment zone – provide the context for assessing the feasibility of eradication. This framework does not require in-depth analysis of many population dynamics parameters such as fecundity, recruitment, or carrying capacity. The consistent critical information required to estimate two of the three components, number of passes and time window, relate to maturation time. If, and only if, the entire system cannot be treated within the time window, knowledge of spread is also needed, to estimate treatment zone. Additionally, if, and only if, adults cannot be effectively removed, adult survivorship becomes relevant because we need to know how long adults will continue to produce new individuals. Nevertheless, our framework dramatically reduces information requirements, thereby allowing more rapid management action.

As with any eradication effort, there is a chance that some individuals will be missed. To evaluate success, monitoring should follow any eradication effort. Arguably, populations missed during the eradication program should be limited to sparse aggregations of individuals (or “nascent foci” as per Moody and Mack 1988; Mack and Lonsdale 2002). Monitoring could focus continued eradication efforts on these sparse populations and treat them locally, rather than restarting the entire eradication program. Furthermore, with smaller population sizes, demographic stochasticity and Allee effects can result in the extinction of remaining individuals (Liebhold and Bascompte 2003).

**Application to Ciona intestinalis in Prince Edward Island**

Ciona is a major nuisance in PEI, with large numbers of individuals and high fecundity making them appear, at first glance, to be impossible to eradicate. However, eradication may be possible, and relatively little information is required to assess its feasibility. The feasibility of eradication is increased by the fact that Ciona does not survive everywhere – it requires solid substrata, which, in PEI, primarily consist of man-made structures, such as docks, buoys, mussel farming infrastructure, and mussels themselves. Ciona have been observed on rocks in their native range (Dybern 1965), but current evidence suggests that they should be very sparse or absent on rocks in PEI, as there is virtually no natural surface available for settlement (Locke et al. 2006). The available substrates are predominantly silt, mud, or sand (Cambell 1973;...
Eradication of nuisance species
PK Edwards and B Leung

MacWilliams and Judson 1973; Murchison 1973), which are unsuitable for colonization by *Ciona*.

At the local scale, eradication could be very effective were the removal of individuals to be conducted in one of three ways, depending on the type of surface targeted. First, vinegar is 100% effective against *Ciona* after one minute of exposure (Carver et al. 2003). Second, air drying is also completely effective, and either of these methods could be used to clear any structure that can be removed from the water (eg mussel lines, buoys, small docks). Larvae that settle after the object is returned to the water could be removed in a second pass. For these structures, the vulnerability time window would be the minimum maturation time, estimated at 45 days (Liu et al. 2006).

Finally, all permanently installed structures and any natural substrata found to support *Ciona* could be cleared, either by hand or by vacuuming (Coutts 2002). Removed individuals are unable to re-attach and should die off quickly on the silt bottom. While this method requires considerably more effort, it is still highly effective against individuals above a visible size (Pannel and Coutts 2007).

We have estimated the total cost of treating each of these surfaces, including mussel farming equipment and docks, as follows: ~ CDN$1.1 million (1.00 Canadian dollar = ~ 0.998 US dollars) per pass for mussel farming infrastructure, and ~ CDN$561 000 per pass for docks and other man-made structures (WebPanel 1).

If coordinated treatments could be completed on these surfaces within the time window, recruitment would be stopped and the cycle would be disrupted. The number of passes required for this to occur can be estimated for *Ciona* using the following equation:

\[
P = \text{CEIL} \left\{ \frac{t_v + L + N_t \cdot t_v}{\text{tw} - \text{tt}} \right\} + 1
\]

where the CEIL function rounds its parameter up to the nearest whole number, \( t_v \) is the visible age, \( L \) is the maximum larval duration, \( N_t \) is the number of populations treated, \( t_t \) is treatment time of a local population (eg a single dock), and \( t_v \) is the duration of the time window. For manual treatment, the first pass, represented by the “+ 1”, removes all *Ciona* of visible size, including adults. Thus, after the first pass, larvae are no longer produced, and only non-visible juveniles and larvae remain (\( t_v + L \)). The \( N_t \) term expresses the time spent conducting the first pass, during which larvae would continue to be generated, recolonizing structures. At each additional pass, juveniles that had grown to visible size would enter the window and could be eradicated. Thus, we can determine the number of passes required by dividing the surviving untreated stages – previously invisible ages, larvae, and individuals that matured during treatment – by the window. The term \( t_v \) is included in the denominator of our equation to account for the fact that individuals will age during treatment of a local area. For treatment with vinegar or air, all attached individuals are killed and \( t_v \) would effectively equal zero. Given the equation and the length

**Figure 3.** The “treatment zone” concept over many populations: (a) at the initial time interval, populations B, C, and D are out of the range of spread from untreated sources; (b) at the second time interval, after treatment passes on populations B–F, populations B, C, and D (designated by X) are cleared, and the treatment zone is moved to encompass new overlapping populations; A, E, and F are now out of range of spread from untreated sources. It may be possible to sweep through a system, eventually allowing full eradication, if the treatment zone exceeds the maximum dispersal distance, and if each pass across local populations within the treatment zone can be completed within the vulnerability time window (Equation 1).

---

**www.frontiersinecology.org** © The Ecological Society of America
of the larval phase (1.5 days), taking $t_e$ to be the difference between visible age (25 days) and mature age (45 days), and assuming that each of the five infested bays has 12 small docks which can be treated in a day ($t_t = 1$ day), we estimate four passes would be needed for manually-treated structures, and two for air- or vinegar-treated ones.

In summary, we believe that eradication may be possible if treatments can occur within a time window of ~20 days for manually treated structures (man-made and rocky structures) and ~45 days for vinegar-treated structures (mussel-farming infrastructure). We provide guidance for research: the most important information needed to assess the feasibility of eradication relates to maturation time, which will dictate the time window. In addition, we emphasize the importance of coordination and of multiple passes.

**Benefit–cost ratio of eradication**

Since *Ciona* threatens the valuable mussel-farming industry, the potential benefits of eradication are high. To estimate the economic benefits, we calculated the discounted cost of current treatment efforts to be CDN$28 million (at a 5% discount rate). At an estimated cost of CDN$4.4 million, our strategy has a benefit-to-cost ratio of 6.3:1 (see WebPanel 1 for calculations). Put another way, an eradication attempt would be economically worthwhile even if there were only a $\geq 16\%$ chance of success. This estimate implicitly incorporates all possible reasons for eradication failure, such as lack of stakeholder participation or poor coordination of treatment. These cost–benefit estimates provide managers with a lower boundary for the total probability of failure, below which eradication would no longer be economically viable.

If *Ciona* were found to be present in substantial numbers on natural structures, this ratio would become 1.7:1. Nevertheless, eradication in currently infested bays would effectively negate the potential spread to other bays within PEI. The treatment costs for all the mussel-producing bays would be ~ CDN$4.9 million, so that to prevent the potential spread to the other bays in PEI, the value of eradication would have a 4.3:1 benefit-to-cost ratio, even if we needed to treat natural structures. Regardless, our approach provides guidance on when eradication efforts may (or may not) be economically viable, and which data are required to make these decisions.

**Discussion**

Currently, efforts to control *Ciona* are carried out at substantial annual costs, and with no long-term benefits. These efforts have little long-term impact on *Ciona* populations, because management has been largely uncoordinated—individual mussel farmers treat their equipment, but do not synchronize with other farmers (T Landry pers comm). Furthermore, there has been no attempt to treat other structures, such as docks. Although there are few docks, when untreated they still act as sources of new larvae. Scientists can aid society by framing the problem so that potential solutions emerge. We have shown here how eradication could be realized with modifications to existing efforts.

Prior frameworks have developed valuable basic criteria for considering eradication (eg Bomford and O’Brien 1995; Mack et al. 2000; Myers et al. 2000), but more quantitative guidance is needed. Our efforts overlap several of these frameworks, highlighting the need for coordination, continued political will over the entire period of treatment, and the required susceptibility of the organism to treatment. In addition, we offer quantitative results: how many passes are required by how many people, over what time frame and area, and at what cost. We show that multiple passes are needed, even when population levels of the invader are not causing immediate ecological or economic harm. Our framework facilitates rapid assessment and response by focusing research: maturation time, and the associated vulnerability time window, are the most critical components needed for calculating eradication feasibility. Once the extent of the infestation is known, we argue that eradication may be achieved even if only a fraction of pre-reproductive stages are treatable (the vulnerability time-window and ratchet-effect concepts) and even if the system is too large to be treated simultaneously (the treatment-zone concept).

Such treatments could be conducted at the bay scale, to minimize resource demands. In estimating the local rate of spread of *Ciona*, we believe that the bays act as natural barriers. *Ciona* larvae are short-lived and may not be able to survive transfer between bays by natural means, such as tidal currents. The species’ slow historic rate of spread (approximately one bay per year) supports this hypothesis and suggests that inter-bay spread is primarily due to rare, long-distance events via human-mediated vectors. This information suggests that, on the time scale of an eradication effort, influx from external vectors will be very limited. Should a manager wish to treat each bay individually, it may be advisable to implement restrictions on certain activities within the bay; this could further increase the probability of success.

While we believe that eradication may well succeed for *Ciona*, the probability of success only needs to be 16% to be worthwhile, based on cost–benefit analysis, if natural structures are uninfested, as appears to be the case in PEI (Locke et al. 2006). This risk evaluation, in combination with the apparent feasibility of our strategy, suggests that the eradication option has been prematurely dismissed and is worth considering for *Ciona* in PEI. Our framework can be sufficiently generalized to identify possible opportunities for eradication in many other systems, as it was built from core concepts that apply to nearly all invasions. The heart of the framework, the vulnerability time-window concept, whereby a species must have a particular pre-reproductive vulnerable stage, is applicable to many species. Our framework was constructed to tackle the
common problem of new invaders with no associated population data. This is the case with many invaders, and the use of our framework may be a valuable diagnostic tool that can help in formulating a solution.

Although we have presented an in-depth analysis of our framework only for Ciona in PEI, we believe it has broad applicability. It is based on general character traits shared by many invaders and requires minimal information. The gray squirrel (Sciurus carolinensis) in Italy (Bertolino and Genovesi 2003), for example, seems to exhibit characteristics favorable to treatment by our method. It has a vulnerable stage before reproduction, when it is confined to a large, visible nest. It is also well known that squirrels remain stationary at this stage, for a period of many weeks (Moore et al. 1997), during which time treatment could occur. This stage could be targeted in many passes, and the adults would not have to be trapped or killed. Additionally, the range and rate of spread of gray squirrels are well known (Bertolino and Genovesi 2003).

While our framework offers one conceptual avenue for eradication, others may be possible. We urge researchers to further develop a suite of general frameworks to fully identify the scenarios where eradication may be possible, and to quantitatively assess the feasibility and the conditions necessary to achieve eradication, using minimal population data. Conversely, these frameworks can also clarify when we should expect eradication efforts to fail. For example, if treatment cannot be completed within the time window, or managers only attempt a single eradication pass, the cycle will probably remain unbroken and eradication efforts will be ineffectual. Similar frameworks should be developed for eradication as well as in other avenues of invasive species management, such as mitigation or prevention.

Conclusions

Our framework provides practical, timely, quantitative guidance for evaluating the strategy that will be of most use to managers: cost, required number of workers, timing of treatments, spatial scope, and number of passes – all with very little population data. We demonstrate its power by taking an invasion once considered hopeless and showing how, when seen from a new perspective, full eradication not only seems feasible, but may actually be economically preferable. Thus, eradication should be seriously considered and quantitatively analyzed. We hope that this perspective will be used for other invasions and that eradication will prove feasible where, historically, it was considered impossible.

Acknowledgements

The authors wish to thank T Landry (Canadian Department of Fisheries and Oceans) and G Arsenault and J Davidson (both University of Prince Edward Island), for all of their local support throughout this project. Additional thanks to L Johnson for insightful comments on the manuscript. Funding for this project was provided by the Canadian Aquatic Invasive Species Network (CAISN).

References


DFO (Department of Fisheries and Oceans, Canada). 2006. An economic analysis of the mussel industry in Prince Edward Island–Gulf region. Moncton, Canada: DFO Policy and Economics Branch, Gulf Region.


McDonald C. 2003. What is the tunicate situation in Atlantic Canada? Proceedings of the Atlantic Canadian Tunicate Workshop; 2003 Mar; Charlottetown, PEI. Ottawa, Canada: DFO. Aquaculture Collaborative Research and Development Program report MG-02-11-003.


Below, we attempt to make estimates of the costs involved in our eradication strategy. We have separated the costs by type of structure. The numbers presented are based on our own estimates and those made by academics and government scientists in Prince Edward Island (PEI). All monetary figures are given in Canadian dollars (CDN$1.00 = ~ US$0.998).

### Docks

For estimates, we took every dock to have three supporting poles, 35 cm in diameter, located every 3 m (J Morriscey pers comm). Another solid form of dock, commonly called a Berlin Wall dock, would have a comparable surface area as the pole-supported dock. The largest of the docks, located in Montague, with an estimated 150 poles (G Arsenaault pers comm), was taken as the upper boundary for size of main docks; using this as an analogue for each bay’s main dock is an overestimate. Each bay has only a few smaller docks, each taken to be 30 m in length. Again taking the Montague River as the model, each bay has 10 small, personal structures, 10 m in length. This gives a total area on the order of 3000 m² per bay. For five infested bays, the total is 15 000 m². The clearance rate was estimated at 4 m² hr⁻¹. This yields 750 hours per bay. At 4 hours per diver-shift, this would be 187 diver-shifts per bay to clear all public and private docks. A team of 10 divers could therefore clear all docks and wharfs in a bay in the 20-day time window. At an estimated $600 per diver shift, the total cost for all five infested bays would be $561 000 per pass.

A voluntary removal order for all temporary structures, such as mooring buoys, floating wharfs, and swimming docks, would both decrease the cost and increase the effectiveness of the effort. Most of the smaller docks could be removed from the water with the cooperation of owners.

### Lines and socks

Based on information from mussel harvesting operators, the costs of boat fuel and labor per longline were estimated to be $100 and $150, respectively (J Davidson pers comm). Based on 200 socks per line (J Davidson pers comm), $0.40 per liter of vinegar, and 1 liter per sock sprayed, each line would require approximately $80 worth of vinegar. This brings the total for spraying to $330 per longline. We estimated this to be much less expensive than dipping and equally effective (Carver et al. 2003). There are a total of 11 110 mussel lines, as estimated from the DFO economic analysis. Thirty-one percent of these lines are in infested areas (DFO 2006), so the total cost of treating all infested lines would be $1.1 million each for the two passes required. The supporting lines and buoys themselves could be treated in concert with the socks, since they too must be lifted with the socks. This would be expected to add little to the cost or time required.

### Natural structures

From GIS data, the area of the infested bays (minus the newly infested Murray Harbour) is 67 km² (Cambell 1973; MacWilliams and Judson 1973; Murchison 1973). The described area contains several different substrata. *Ciona* may settle on rocky substrata (2.53 km²), but are probably sparse to absent in PEI (Locke et al. 2006). Given this sparseness, scouring would be limited mainly by swim and search speed, because *Ciona* would require little handling time to clear. We estimated diver swim speeds of 0.125 km hr⁻¹ to search every centimeter of the rocky substrate. Based on a 1-m swath of viewing, we estimated 5060 diver shifts, for a total of $3 million per pass. This effort would need to be conducted within the same time window as other structures, and would require as many passes as a dock. Based on these numbers, a team of 126 divers could achieve this in a 20-day time window. Given the ranges of this estimate, search rate should be validated before an eradication effort is started. While this would undoubtedly be a major effort, it is not unreasonable, given the potential gains.

We estimate the complete eradication to cost $4.4 million; if natural substrates are infested, this could increase to $16.4 million.

### Cost-benefit calculations

By conducting a cost-benefit comparison we can estimate the potential gains of an eradication effort or estimate the required probability of success for eradication. Alternatively, we can calculate how long the invaders must be kept away for the management to remain financially reasonable. A case study that incorporates additional fuel, disposal, labor, and processing fees to eradicate tunicates estimates a current $0.242 additional cost per kilogram of mussels (McDonald 2003). Based on production weight in the infested bays of 5575 metric tons, annual spending to control *Ciona* in the infested bays totals approximately $1.4 million. Therefore, the estimated cost of continued treatment is:

\[
c = \sum_{t=0}^{\infty} \frac{S_1 400 000}{(1 + 0.05)^t} = $28 000 000
\]

where \(i\) is the number of years the treatment is applied, and \(c\) is the total cost, using a 5% discount rate, as is typically done in economic analyses. Discounting can be applied to estimate present-day value of future money. Thus, the benefit-to-cost ratio is $28 000 000 to $4 400 000, or a 6.3:1 ratio. According to our estimate, the eradication effort will cost $4.4 million. Put another way, eradication would also be optimal if there were a ≥ 16% chance of eradication or if it could keep *Ciona* at a level at which they had no impact on industry for 4 years.
WebPanel 1. Costs – continued

References

DFO (Department of Fisheries and Oceans, Canada). 2006. An economic analysis of the mussel industry in Prince Edward Island–Gulf region. Moncton, Canada: DFO Policy and Economics Branch, Gulf Region.
McDonald C. 2003. What is the tunicate situation in Atlantic Canada? Proceedings of the Atlantic Canadian Tunicate Workshop; 2003 Mar; Charlottetown, PEI. Ottawa, Canada: DFO. Aquaculture Collaborative Research and Development Program report MG-02-11-003.