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POTENTIAL OF ABALONE SHELLS AS VECTORS FOR EXOTIC OYSTER SPECIES IN THE ABALONE FARMING PRACTICE IN CHINA

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ABSTRACT Pacific abalone *Haliotis discus hannai* (Ino, 1953) aquaculture is a thriving and prosperous industry in China, producing more than 110,000 metric tons in 2013. In recent years, Fujian in the southern region of China has become the chief abalone farming center, accounting for 85% of the total Pacific abalone yield. The practice of transferring abalone to northern regions to oversummer emerged because of challenges in Fujian, such as fouling, high temperature stress, and unexpected extreme weather events. From the view of ecology and conservation biology, however, the potential ecological risks of this practice should be considered as the abalone shells could act as vectors of exotic organisms. In this study, oyster samples from the shells of live, farmed abalones were collected in Fujian in southern and Rongcheng in northern China. The oysters were identified using a recently developed molecular method. In addition, the fouling oysters on Pacific abalone transferred to the northern region were monitored in a field trial. Survivorship and growth performance of the fouling oysters on the transferred abalones were determined through investigation of digital images taken at 2-wk intervals during the oversummering period. Results of the molecular analysis showed that fouling oysters collected from southern and northern regions are *Crassostrea angulata* and *Crassostrea gigas*, respectively. The field trial demonstrated that approximately half of the *C. angulata*, from the southern region, were still alive after a 6-mo oversummering period in the northern region. Findings from this study have important implications for aquaculture, the management and monitoring of cultured populations of Pacific abalone, and the conservation of wild oyster species in China.

KEY WORDS: Pacific abalone, *Haliotis discus hannai*, *Crassostrea*, bio-sanitation

INTRODUCTION

Pacific abalone *Haliotis discus hannai* aquaculture is a thriving and prosperous industry in China, producing more than 110,000 metric tons in 2013 (China Bureau of Fisheries 2014). Two innovations are considered to have contributed to the booming industry. One is the application of positive heterosis from crossbreeding between stocks from Japan and China (Zhang et al. 2004, Deng et al. 2008), and the other is the improvement in sea-based aquaculture technologies, including abalone long-distance transport techniques in sea-based farming systems (Wu et al. 2009). Since the beginning of the century, the transfer of abalone seeds from northern regions for overwintering in warmer waters of the southern regions has become a routine practice in China. Furthermore, rapid development of technologies in both seed production and sea-based culture in local farms has shifted the farming center of the industry from Shandong and Dalian (northern regions) to Fujian (a southern region). Although innovative culture methods, such as cage culture, have immerged in recent years (Wu & Zhang 2013), the majority of sea-based production is still conducted with suspended six-tier baskets, especially for small-scale abalone farms (Wu et al. 2009). The sea-based multitier basket culture of *H. discus hannai* was developed from a land-based farming system for small abalone *Haliotis diversicolor supertexta* (Lischke, 1870) (Chen & Lee 1999).

There are still problems in local abalone farming in Fujian. Fouling, high temperature stress, and unexpected extreme weather events (such as typhoons and red tides in the summers) pose great risks to local sea-based abalone farms (Wu & Zhang 2010). According to several abalone production corporations, one way they have avoided these potential risks in recent years is by transferring 1-y-old juveniles and adult abalones to northern regions for oversummering. Before transfer, removal of fouling organisms is usually managed by manually removing abalones from the baskets, brushing soft fouling organisms away, and killing sessile fouling organisms. Plastic multitier baskets for holding abalones are also exchanged with clean baskets of the same type and size. This manual bio-sanitation method is believed to be feasible based on the high value of the reared animals and low labor cost in local regions.

Despite this bio-sanitation practice of manually removing fouling organisms, an ecological risk assessment of the transfer farming method in China has not yet been conducted. Compared with soft fouling organisms such as clionid sponges and ascidians, sessile fouling organisms such as oysters are difficult to remove. A small-cupped oyster, the exact species unknown, has become the main sessile fouling organism in both the northern and southern farming regions. Molecular barcodes for oyster species identification were previously proposed according to sequence polymorphisms of the mitochondrial *16S rRNA* gene, cytochrome oxidase I (*COI*), and nuclear *28S rRNA* (Wang & Guo 2008, Wang et al. 2010). A multiplex species-specific polymerase chain reaction (PCR) method for oyster classification has also recently been published (Wang & Guo 2008). According to Wang and Guo (2008), the small-cupped oyster from southern China was *Crassostrea angulata*, and was separated from another species of small-cupped oyster *Crassostrea gigas* in northern China by the Yangtze River. If

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this finding is true, it is possible that abalone shells are vectors for exotic oyster species due to the interregional culture practice in China. No large-scale survey has yet been conducted here, resulting in uncertainty regarding the introduction of exotic oysters by transferred abalone interregional and the survivorship of any potentially introduced oysters.

In this study, fouling oyster samples from live cultured abalone were collected from local abalone farms in the northern and southern regions of China and examined using a previously published molecular method. A field trial was also conducted to assess growth and survival of the potentially introduced oysters based on the results of fouling oyster species identification. The objective was to determine whether abalone shells act as vectors of exotic oyster species in the interregional culture practice in China and whether introduced fouling oysters can survive during the summer. Suggestions for local legislation and more rigorous bio-sanitary procedures and recommendations for future abalone aquaculture practice are also discussed.

MATERIALS AND METHODS

Sample Collection and Fouling Oyster Species Identification

Samples of fouling oysters attached to abalone shells were collected from April to October 2010 in three locations in southern China: Dongpu, Putian (DP, 25° 07′ 18.89″ N, 119° 02′ 16.73″ E), Changyao, Ningde (CY, 26° 33′ 35.90″ N, 119° 49′ 45.31″ E), and Gulei, Zhangzhou (GL, 23° 45′ 24.31″ N, 117° 35′ 16.65″ E), and one location in northern China: Lidao, Rongcheng (LD, 37° 13′ 1.70″ N, 122° 37′ 47.67″ E; Fig. 1). The sampling dates and sites were selected in accordance with standard industry practices for interregional culture (Table 1).

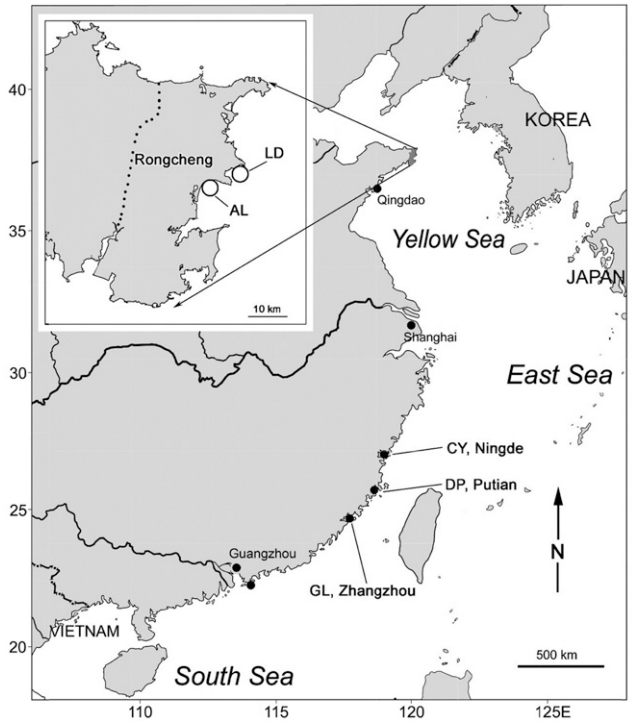

Figure 1. A map of coastal China showing the collection sites of oyster samples and abalone farming regions.

At each site, fouling oysters of all sizes that were attached to abalone shells were collected at random. Further, any wild or farmed oysters in sea-based systems occurring in the fouling oyster collection sites were also sampled for analysis regarding the possible origins of fouling oysters.

With respect to fouling oyster species classification, a phylogenetic analysis of the *COI* and *16S* sequences was conducted as described in the work of Wang and Guo (2008) with some modifications. Deoxyribonucleic acid was extracted from the ethanol-fixed adductor muscle of oysters with a shell length (SL) greater than 2 cm or else from other soft parts of oysters with SL less than 2 cm using a Qiagen DNeasy kit. Polymerase chain reaction was performed on 25 ml solution using a PE GeneAmp 9700 Thermal Cycler. The PCR conditions were optimized by testing different primers, Mg^{2+} concentrations, and annealing temperatures. Primers (Promega) 16sar and 16sbr were used to amplify segments of the mitochondrial *16S rRNA* gene. Segments of *COI* were amplified with primers LCO1490 and HCO2198. The *COI* and *16S* sequences were obtained with an ABI PRISM 377XL DNA Sequencer, and these sequences together with *Crassostrea* spp. sequences downloaded from GenBank were subjected to phylogenetic analyses to determine the oyster species (Wang & Guo 2008).

Field Monitoring and Potential Growth and Survival of Exotic Oysters

A field trial in the summer of 2011 was conducted to assess whether abalones can act as vectors of exotic oysters from southern to northern sea-based abalone farms in China. In addition, detailed investigations were carried out to monitor growth and survivorship of the fouling oysters in an abalone stock. In May 2011, an abalone stock transferred for oversummering from a sea-based farm in Ningde, southern China, was divided into two groups with one group relocated to Ailian Bay and the other to Lidao Bay, both in Rongcheng, northern China (Fig. 1). Immediately after transfer to Rongcheng, the abalones were carefully examined for fouling oysters. Although manual removal of oysters was conducted in Ningde before transfer, several oysters on the transferred abalones were visually discovered by the farmer. A total of 150 abalones with fouling oysters from each site were collected and tagged individually with numbered beads glued to the outer shell (Kube et al. 2007, Wu et al. 2013). The tagged abalones were partitioned into three equal replicates and reared in accordance with local farming practices.

Digital images were taken of the abalones to document the location of the identifying tag and the distribution of the fouling oysters on the outer shells (Fig. 2). Digital images were also used to distinguish newly recruited oysters (referred to as ''recruited oysters'' in the rest of the document) that established on the abalones from the existing transferred oysters (referred to as ''transferred oysters'' in the rest of the document). New images were taken once every 2 wk from May 10, 2011, to the termination of the field trial on November 10, 2011.

To determine survivorship of the transferred oysters from southern regions, Kaplan–Meier estimates of survival (Kaplan & Meier 1958) were plotted using data gathered from each digital image. To compare survival curves of fouling oysters between the two northern sites, nonparametric univariate log-rank and Wilcoxon rank-sum tests were conducted (significance level of 0.05). Neither log-rank nor Wilcoxon rank-sum tests

TABLE 1.

Sampling location, origin, date, and species of fouling oysters collected from abalone reared in interregional culture facilities.

Location	Origin	*n*	SL (mm)	Sampling date	Oyster species
DP (S)	Fouling	117	13–35	April 24, 2010	*Crassostrea angulata*
	Wild	50	10–55	April 24, 2010	*C. angulata*
CY (S)	Fouling	86	12–40	April 28, 2010	*C. angulata*
	Wild	50	10–55	April 28, 2010	*C. angulata*(26)/*Crassostrea sikamea* (24)
	Cultured	44	35–82	April 28, 2010	*C. angulata*
GL (S)	Fouling	35	12–50	April 21, 2010	*C. angulata*
	Wild	50	10–55	April 21, 2010	*C. angulata*
	Cultured	50	40–95	April 21, 2010	*C. angulata*
LD (N)	Fouling	55	13–50	October 16, 2010	*Crassostrea gigas*
	Wild	39	12–60	October 16, 2010	*C. gigas*
	Cultured	78	55–140	October 16, 2010	*C. gigas*

S, sampling location in the southern regions; N, sampling location in the northern region.

assume any particular distribution of the survivor curve, and where the log-rank test places more weight on later survival times, the Wilcoxon rank-sum test places more weight on early survival times. Differences in abundance of newly recruited oysters between the two sites were analyzed with a rank-sum test (significance level of 0.05). All data were analyzed using IBM SPSS Statistical Software (version 19.0).

Initial and final shell sizes of the transferred oysters were also recorded in the field trial. The measurements were taken using electronic vernier calipers with an accuracy of 0.01 mm.

At the termination of the field trial, 50 transferred and 50 recruited oysters from each site were randomly selected for species identification using the abovementioned molecular methods.

RESULTS

Fouling Oyster Species Identification

A total of 293 fouling oysters on farmed abalone from southern (DP, CY, and GL) and northern (LD) regions were collected (Table 1). In addition, another 189 oysters from wild populations and 172 from sea-based farms were collected from the same locations. All oysters collected were successfully identified by the haplotype polymorphisms of the *16S rRNA* gene and mitochondrial *COI* DNA sequences. Fouling oysters collected on living Pacific abalone shells from the southern regions of China were all *Crassostrea angulata*. Similarly, nearly all oysters sampled from the wild populations and sea-based farms in the southern regions were *C. angulata*. One wild population in CY, Ningde, however, was an exception in that approximately half of the oysters were *C. angulata* while the other half were *Crassostrea sikamea* (26 versus 24, respectively). In terms of oyster identification in the northern region, all oysters sampled from LD, Rongcheng, including fouling, wild, and farmed oysters, were *Crassostrea gigas*. It is clear that oyster species varied between abalone farms in northern and southern regions.

Figure 2. Digital images of abalone shells showing the locations of identifying tags and fouling oysters.

Fouling Oyster Survivorship and Growth Performance in Northern Regions

A total of 336 and 346 oysters were transferred from the southern regions to Ailian and Lidao Bays, respectively, on the outer shells of farmed Pacific abalone. Survival analysis showed that mean survival of all fouling oysters was 48.1% after 6 mo of oversummering. No significant difference was detected in survival of the transferred oysters between the two locations (Wilcoxon rank-sum test, $P = 0.46$; Fig. 3A).Without exception, all transferred fouling oysters collected in the beginning and end of the field trial were *Crassostrea angulata*. In contrast, the newly recruited fouling oysters were identified as *Crassostrea gigas*.

A large increase in abundance of newly recruited fouling oysters occurred in early to late August in Ailian Bay (Fig. 3B). The digital images showed 95 oysters fouling 150 abalones in Ailian Bay; however, recruitment was different in Lidao Bay where only 12 oysters were recruited. Because of the large difference in the number of newly recruited oysters between the two locations, the statistical comparison was omitted due to not having sufficient power to perform the test.

By the end of the field trial, SL of the transferred fouling oysters increased approximately 1 cm from 3.59 ± 1.29 to 4.27 ± 1.47 cm (Fig. 3C), and the newly recruited *Crassostrea gigas*

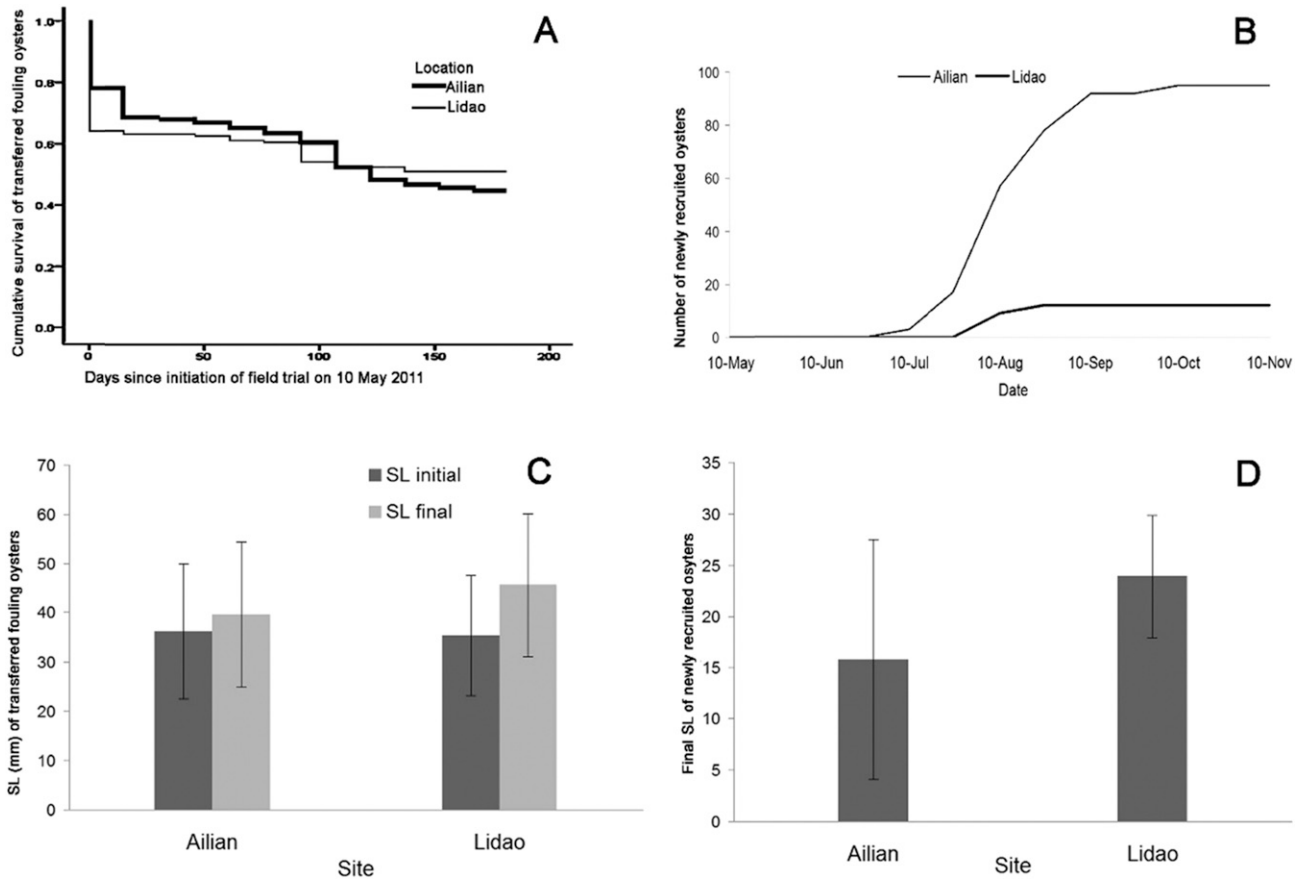

Figure 3. Survival and growth performance of fouling oysters on abalone transferred from southern China (Ningde) to northern China (Ailian and Lidao Bays). (A) Survival curves for oysters transferred with the abalones from Ningde to Ailian and Lidao Bays, (B) abundance of newly recruited fouling oysters on transferred abalones in Ailian and Lidao Bays, (C) initial and final SL of transferred fouling oysters in Ailian and Lidao Bays, and (D) final SL of the newly recruited fouling oysters in Ailian and Lidao Bays. Survival, abundance, and SL were determined from digital images taken of the abalones once every 2 wk from 10 May to 10 November 2010.

oysters obtained a SL of approximately 2 cm from 1.58 ± 1.17 to 2.38 ± 0.60 cm (Fig. 3D), though, variance was high.

DISCUSSION

Shells of molluscs as potential vectors for the introduction of exotic organisms has raised the concerns of ecologists, geneticists, and conservation biologists over a long time (Cohen & Zabin 2009). It is believed that the introduction of animals brings genetic and ecological risks to local biodiversity and possibly the transmission of pathogens. A variety of organisms attach to the shells of molluscs, including algae, protozoans, sponges, hydroids, anemones, serpulid worms, limpets, mussels, barnacles, tanaids, amphipods, tunicates, and others. Other unattached organisms, such as some worms, can also be transported on molluscs, and molluscs and crustaceans have been shown to transfer various organisms between sites (see the review of Fitridge et al. 2012). Although documenting the effects of entire fouling communities is important, identifying the species primarily responsible for negative impacts can aid in tailoring management strategies to the removal of these species (Sievers et al. 2013). In terms of Pacific abalone farming in China, oyster fouling is the primary concern to abalone sea-based farmers because it is challenging to remove oysters. In this study, it was confirmed that fouling oysters in southern regions can be transferred with Pacific abalone to the northern regions despite application of the bio-sanitation practice of manually removing fouling organisms before transfer.

Oyster identification is problematic in China because of the varying sizes, different shapes, and widespread distribution of the animals. Recent advances in molecular methods available for oyster classification, however, have solved this problem (Wang & Guo 2008). According to recent reports, the small-cupped oysters collected from wild populations in southern China were *Crassostrea angulata*, whereas those from north of the Yangtze River were *Crassostrea gigas* (Wang et al. 2010). The present study confirmed this distinction. The oysters transferred from southern regions were all *C. angulata*, whereas those recruited in the north were all *C. gigas*.

In this study, digital images were analyzed to distinguish transferred oysters from newly recruited ones. On the basis of these images and molecular evidence, it was concluded that abalones can and do act as vectors of exotic oysters. In addition to the conclusion that abalone shells are vectors of exotic oysters, three other findings demonstrate potential ecological risks of exotic oysters introduced from the southern to the northern regions of China. First, the species of oyster in the

southern regions is different from that in the northern regions. Molecular evidence showed that the oysters sampled from fouling, wild, and farmed stocks were *Crassostrea angulata*, with the exception of some *Crassostrea sikamea* from a wild oyster population in Ningde, Fujian. Without exception, all oysters from the northern region were all *Crassostrea gigas*. Second, the oysters transferred from southern regions were *C. angulata*, which is unlikely to be naturally distributed in the northern regions. Finally, the transferred fouling oysters, *C. angulata*, were able to survive after a 6-mo oversummering period in the northern region. About half of the *C. angulata* attached in the outer shells of Pacific abalone in both of the northern sites, Ailian and Lidao Bays, were alive at the termination of the field trial.

Large initial mortality of transferred fouling oysters *Crassostrea angulata* occurred both in Ailian and Lidao Bays, Rongcheng, northern China (Fig. 3A). The potential reason is probably the poor acclimation of this species due to the cold environment in Rongcheng. When the transfer of Pacific abalone together with fouling *C. angulata* begins in the early May every year, the ambient water temperature in Rongcheng is 10°C lower than that in bays of Fujian, the native habitat of *C. angulata*. After the initial mass mortality, the transferred fouling oyster *C. angulata* can survive during the oversummering (Fig. 3A). There are, however, no *C. angulata* found in the wild at the northern sites reported in this study (Table 1) or other publications in recent years due to the occurrence of abalone interregional culture practice. One possible reason is the small abundance of the transferred *C. angulata*. The fouling oysters have detrimental effects on the appearance and marketability and on the growth and condition of farmed animals (Fitridge et al. 2012). Since the fouling oysters are challenges to Pacific abalone farmers, the farmers manage to remove the fouling oysters in the farming practice. The other reason is that it is probably tough for *C. angulata* to overwintering in northern China due to the low water temperature in the winter days.

The abundance of newly recruited oysters *Crassostrea gigas* at the two sites of Ailian and Lidao was different as shown in Figure 3B. Potential reason for the difference is probably the current blocked by the land as shown in Figure 1 poses impacts on oyster *C. gigas* recruitment. Actually, the situation of oyster recruitment between the sites of Ailian and Lidao is varying year by year according to observations of the local farmers, although the two sites is very close of less than 5 km. More investigations about the *C. gigas* recruitment should be carried out in the further study. Compared with the spread of *Crassostrea angulata* in northern sites, the spread of *C. gigas* to southern regions might be of less concern because the practice of transfer from northern to southern sites is much less than the inverse operation. The possible spread of *C. gigas* to southern sites, however, still requires investigations from the viewpoints of ecologists and conservation geneticists.

This study demonstrated the transfer of *Crassostrea angulata* via abalone shells from the southern to the northern sites. The key research questions that might be addressed as follow-up to this study are whether *C. angulata* can reach reproductive maturity during summer. It is important for evaluating whether the oysters may be spawning in the abalone interregional farming practice, and whether the possible parasites or pathogens via fouling oysters will be threats to local oyster species in northern sites should also be the potential concerns with the transfer of *C. angulata* in the future study.

Although there is no evidence in this study that exotic *Crassostrea angulata* pose an ecological risk such as through hybridization with local species or undergo biological events such as spawning, the Pacific abalone interregional farming practice should be carefully reevaluated. Recommended practices considered for the protocols to prevent transfer of exotic oysters are reported through the avoidance of natural recruitment, physical removal of oysters, and the use of antifoulants before transfer (Fitridge et al. 2012). More rigorous biosanitary procedures to the issue of animal interregional farming are the prohibitions by legislation after detailed investigations and scientific researches such as the occurrence in developed countries. Rapidly growing interest in interregional farming in the abalone industry, however, suggests that the quantity of fouling organisms transported is likely to increase. Before the transfers of fouling organisms via hitchhiking on Pacific abalone increases much further, it would be wise to conduct the research needed to develop protocols that will prevent further interregional transfer of exotic organisms.

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