2011

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Recommended Citation

Mattei, J., Beekey, M.A., Potter, H.R., Bond, C.S., Woronik, A., Roberts, J.A., & Smith, K.A. (2011). Estimation of short-term tag-induced mortality in horseshoe crabs Limulus polyphemus. Transactions of the American Fisheries Society, 140(4), 954-958. doi: 10.1080/00028487.2011.601223

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Transactions of the American Fisheries Society 140:954–958, 2011
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ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2011.601223

ARTICLE

Estimation of Short-Term Tag-Induced Mortality in Horseshoe Crabs *Limulus polyphemus*

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Abstract
Horseshoe crabs *Limulus polyphemus* range along the East Coast of the United States and over 150,000 of them have been marked with U.S. Fish and Wildlife Service disk tags. It has been assumed that the tags do not harm the animals and are similar to common epibionts often found on the shells of horseshoe crabs. We investigated whether newly tagged adult female horseshoe crabs would exhibit higher short-term mortality rates than untagged adult females. All crabs were collected from a beach in Connecticut and then were transported to a laboratory for the experiment. Tagging involved drilling a small hole through the carapace in the lower back corner of the prosoma and then inserting the tag into the hole. Overall mortality of tagged and untagged females held in flow-through raceways for 44 d was minimal (0% mortality among 53 tagged crabs; 4% mortality among 52 untagged crabs). None of the horseshoe crabs lost or shed their disk tags over the course of the experiment. In field mark–recapture studies of horseshoe crabs, typically between 11% and 20% of the initial numbers of tagged crabs are recaptured. Crabs that are recaptured but reported dead are unlikely to have died from the initial tagging process. Our results indicate that newly tagged adult horseshoe crabs have the potential to survive as well as untagged crabs through the Connecticut spawning season (~30–45 d). Recapture data also suggest that these crabs can survive for as long as 8–10 years with the tags in place.

The horseshoe crab *Limulus polyphemus* is an economically important species because it is harvested for use as bait and for its blood products, which are used in the medical industry (Berkson and Shuster 1999; Manion et al. 2000). Horseshoe crabs are also ecologically important as habitat for other species and as prey (Castro and Myers 1993; Grant 2001). Therefore, federal, state, and nonprofit conservation organizations in the USA seek to conserve horseshoe crab populations and to ensure that this species is sustainably harvested while maintaining its important ecological functions (Niles et al. 2009).

Numerous studies have been conducted to better understand horseshoe crab population dynamics and life history characteristics (Tanacredi et al. 2009; Chabot and Watson 2010). One technique that is commonly used by ecologists is to tag or mark the animals in a population to determine migratory patterns, movement between spawning areas and feeding areas, geographical limitations, and population size; such information is used to develop better management plans (Schwarz and Seber 1999; Swan 2005; Smith et al. 2006). In 1999, the U.S. Fish and Wildlife Service (USFWS) initiated a tagging program by distributing uniquely numbered, round plastic disk tags to mark a proportion of spawning adult horseshoe crabs from different areas. Crabs are tagged off the Atlantic coast from Massachusetts to Georgia. Only mature, spawning adult horseshoe crabs are tagged by the USFWS tagging program (USGS 2009). Smith et al. (2009) documented that both male and female horseshoe crabs reach a terminal molt at maturity. Therefore, tag-induced mortality associated with molting of horseshoe crabs is not a concern.

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Received September 6, 2010; accepted February 11, 2011
Published online July 27, 2011

Since 1999, over 150,000 horseshoe crabs have been tagged. Studies that use USFWS tags have been conducted by a variety of groups including biomedical companies, universities, state and federal agencies, and nonprofit environmental conservation organizations (Shelia Eyler, USFWS, personal communication). Researchers and volunteers of Project *Limulus* have been tagging horseshoe crabs in Connecticut, Rhode Island, and New York for the past 10 years. Based in Long Island Sound, Project *Limulus* is a horseshoe crab research program that encourages citizen participation; the project was founded by and is run by faculty in the Department of Biology at Sacred Heart University. Project *Limulus* has tagged more than 54,000 horseshoe crabs by employing both USFWS-issued disk tags and Floy cinch tags (Beekey and Mattei 2008; Mattei et al. 2010).

In any tagging study, it is important to understand how the physical act of tagging may affect the tagged animal's behavior or mortality risk. Among aquatic invertebrates, several studies have assessed the suitability of different tag types for tagging and marking decapods and a few studies have quantified tag-induced mortality. In some cases, capturing, handling, and tagging have been observed to increase mortality by 50% (Marullo et al. 1976; Bennett and Lovewell 1983; Hill and Wassenberg 1985; Montgomery and Gray 1991; Montgomery and Brett 1996).

Given the large number of horseshoe crabs tagged through the USFWS program and given the evidence in the literature for tag-induced mortality among some aquatic arthropods, it is necessary to investigate the potential effects of the initial tagging procedure on horseshoe crab survival. Past studies that have investigated horseshoe crab mortality due to bleeding procedures conducted by the biomedical industry have estimated average mortality at between 5% and 15% (Rudloe 1983; Kurz and James-Pirri 2002; Walls and Berkson 2003). Hurton and Berkson (2006) found an even higher mortality rate of 29% for bled females that experienced stressful handling conditions over a 14-d observation period. Recently, Leschen and Corriea (2010) studied mortality in female horseshoe crabs that were subjected to conditions that more closely mimicked those imposed by the Massachusetts biomedical industry during blood collection. Leschen and Corriea (2010) held the crabs for 17 d posttreatment in recirculating tanks and also found mortality rates ranging from 22% to 29%. However, no data have been published that address the potential for tag-induced mortality. Therefore, we decided to investigate whether the physical act of tagging would result in increased short-term mortality of horseshoe crabs.

METHODS

Adult female horseshoe crabs (N = 106) were hand-collected in 2010 from Sandy Point Beach (41°16′3.29″N, 72°55′31.16″W) in West Haven, Connecticut; 50 individuals were collected on June 4, and 56 individuals were collected on June 5. Females were collected as they were encountered on the beach without preference for the mating behavior (single, paired, or with multiple males) or condition (newly molted or damaged) of the female. We used only females in this study to increase replication and avoid any possible confounding effects of sex on the results. Horseshoe crabs were transported approximately 21 km to the National Oceanic and Atmospheric Administration, Northeast Marine Fisheries Science Center, Milford, Connecticut; the crabs were held within several large tubs in an air-conditioned van during transport. Upon arrival, the crabs were divided equally into four fiberglass, flow-through raceways (labeled A–D; each 9.15 m long × 1.25 m wide × 0.46 m deep). The water level was kept just below the upper rim of the tank. The raceways were located outdoors and were covered with black fiberglass tops. No sand was added to the tanks, but several centimeters of sediment did accumulate in the bottom of the tanks over the course of the experiment. The raceways were supplied with ambient seawater pumped from Milford Harbor. The crabs were allowed to acclimate in the raceways without physical interference for 2 d. The raceways were inspected on June 6, and one dead female was removed from raceway D.

On June 7, the remaining female horseshoe crabs (N = 105) were divided into three groups based on physical appearance and shell condition (1 = new, 2 = moderate, 3 = worn; Duffy et al. 2006). Condition 1 crabs had a newly molted, glossy carapace that was in excellent condition and free of barnacles and slipper shells. Condition 1 female crabs also had a mucous film over the prosoma, exhibited hairs on the edges of the prosoma and opisthosoma, were light brown in color, and had noticeably shiny compound eyes. Condition 2 crabs were in good physical condition; however, the carapace was scraped or showed other signs of wear or slight damage and was darkened. For condition 2 crabs, the mucous layer on the prosoma was somewhat thinner or less apparent than that observed on condition 1 crabs; condition 2 females were host to a few barnacles and slipper shells and had fewer fringe hairs than condition 1 crabs. In condition 3 crabs, the carapace was very dark with noticeable pitting, and fringe hairs were absent. Condition 3 crabs were also host to many barnacles and slipper shells and generally had missing appendages; they exhibited dents or cracks on the carapace, a heavily worn telson, and damaged or dull compound eyes.

The female crabs were divided randomly, and equal numbers of crabs representing each condition were placed into each raceway (Table 1). A uniquely numbered Floy cinch tag (Model FT-4, 20 cm [8 in]; www.floytag.com) was attached around the last joint of each individual's fifth walking leg (pusher). Prosomal width (PW) was measured to the nearest 0.1 cm for each female. The USFWS-issued disk tags (4.4-cm diameter; each bearing a unique tag number and information for reporting recaptures) were randomly attached to half of the crabs from each condition category within each raceway. Tags were attached to the crabs with a number-2 yellow scratch awl (Challenge Sailcloth, Vernon-Rockville, Connecticut), and a number-8 stainless-steel washer was affixed to the awl with J. B. Weld epoxy that acted as a stopper. A hole (4.0-mm diameter) was created by gently

TABLE 1. Average (±SD) prosomal width (cm) and sample size (in parentheses) for horseshoe crabs in each raceway, treatment group, and shell condition category (1 = new, 2 = moderate, 3 = worn; see additional description in Methods).

Raceway	Treatment	Condition 1	Condition 2	Condition 3	Total N
A	Tagged	28.5 ± 0.5 (3)	28.3 ± 0.9 (4)	24.7 ± 1.5 (6)	
	Untagged	25.2 ± 1.4 (3)	27.3 ± 2.5 (4)	26.1 ± 2.0 (6)	26
B	Tagged	28.3 ± 1.6 (3)	26.4 ± 1.6 (4)	25.7 ± 1.5 (6)	
	Untagged	25.3 ± 1.5 (3)	24.6 ± 0.9 (4)	25.7 ± 1.7 (6)	26
C	Tagged	28.2 ± 1.3 (3)	26.3 ± 2.2 (4)	26.2 ± 2.4 (6)	
	Untagged	26.8 ± 1.9 (3)	27.3 ± 2.8 (4)	25.2 ± 2.0 (6)	26
D	Tagged	26.2 ± 2.4 (3)	26.6 ± 1.7 (4)	25.1 ± 2.4 (7)	
	Untagged	23.3 ± 3.9 (2)	26.8 ± 1.8 (4)	25.1 ± 1.9 (7)	27
Total N		23	32	50	105

twisting the awl into the lower rear of either the right or left posterior side of the prosoma. The disk tag was securely inserted into the hole.

Once per week, the horseshoe crabs were fed ad libitum with herring and squid that were caught in trawls within Long Island Sound. The crabs were given 1–2 d to eat, after which the raceways were cleaned of all leftover food. Each raceway was fitted with a HOBO Pendant temperature data logger that was set to record the temperature every hour. Raceways were checked every weekday for dead crabs.

The experiment ended after 44 d; on July 20, 2010, all crabs were removed from the raceways. The Floy cinch tags were removed from all crabs, and the remaining untagged crabs were fitted with USFWS disk tags. All crabs were transported back to Long Island Sound and were released at the site of capture.

RESULTS

There was no significant difference in survival rates between tagged and untagged horseshoe crabs over the course of the experiment ($\chi^2 = 0.02$, $P \leq 0.80$). By the end of the experiment only two horseshoe crabs had died. These two crabs were in raceway D and were both assigned a condition index of 3. One crab (PW = 29 cm) died on June 28, 2010, and the other (PW = 25.5 cm) died on July 3 or July 4 (weekend days) but was not discovered until July 5. Neither crab had obvious signs of damage to the prosoma, opisthosoma, or telson. However, the smaller crab was noticeably lethargic at the beginning of the experiment. Neither of the crabs that died were tagged with the USFWS disk tag. In fact, all crabs tagged with USFWS disk tags survived for the entire duration of the experiment and were returned to Long Island Sound alive. None of the crabs lost or shed their disk tags over the course of the experiment.

There were no significant differences in PW between tagged and untagged crabs or among condition classes except that condition 3 crabs were significantly smaller than condition 1 or condition 2 crabs (Kruskal–Wallis analysis of variance [ANOVA] by ranks: $P \leq 0.05$; Table 1). Many of the condition 3 crabs used in this experiment exhibited signs of damage (e.g., a dented, cracked, and notched prosoma; missing legs; and a bent telson), and some exhibited signs of lethargy.

The average daily temperature increased over the course of the experiment for all four raceways (Figure 1). There were two temperature drops due to heavy rainstorms on June 6 and June 29, 2010. Average starting temperature across all raceways was 18.1°C, and the ending temperature was 23.3°C. The highest average daily water temperature was 26.2°C, and the lowest was 13.8°C. On June 23, 2010, the temperature logger in raceway C malfunctioned and stopped recording. There was no significant difference between raceways with respect to the average daily temperature recorded prior to the data logger malfunction in raceway C (ANOVA: $F = 0.313$, df $= 91$, $P \leq 0.80$). The average daily temperatures recorded in raceways A, B, and D during the experimental period after the data logger malfunction also did not significantly differ (ANOVA: $F = 0.227$, df $= 149$, $P \leq 0.80$; see Figure 1).

DISCUSSION

Our study showed no tagging-induced mortality among female horseshoe crabs. Only two crabs died in this experiment, neither were tagged. Unlike the bleeding studies conducted previously (Hurton and Berkson 2006; Leschen and Corriea 2010), which demonstrated significant mortality associated with the handling and bleeding of crabs by the biomedical industry, this study provides evidence that the tagging of female horseshoe crabs is a benign process. In captivity, the tagging of an adult female horseshoe crab is unlikely to cause the death of that individual. Once the crab is released into Long Island Sound, tagging is unlikely to cause mortality in the short term. There is a possibility that because the tag is white, it will attract predators. However, there are very few predators of adult horseshoe crabs in Long Island Sound (e.g., large sharks and sea turtles; Walls et al. 2002). The tags do not stay white for very

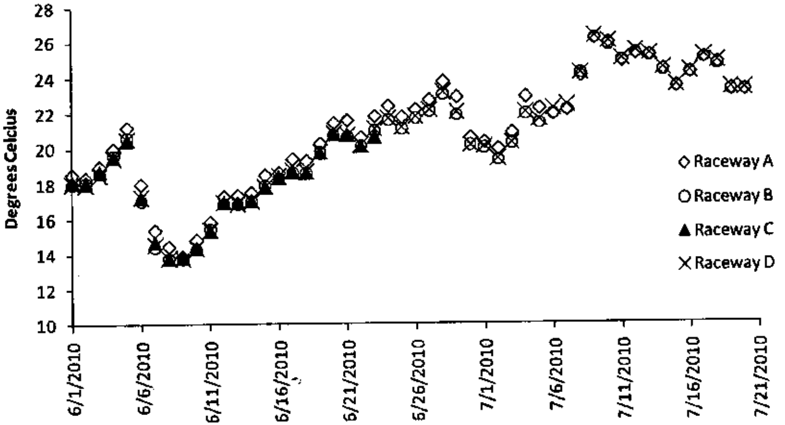

FIGURE 1. Average daily temperatures (°C) for the four raceways in which tagged and untagged female horseshoe crabs were held. The temperature logger in raceway C stopped recording on June 23, 2010.

long; within 6 months to 1 year, most tags on recaptured crabs are encrusted with mud and barnacles. There is no evidence that tagging interferes with spawning; based on the results of this study, tagged crabs should be able to survive over the course of a breeding season (in Connecticut, the majority of spawning takes place from mid-May to the end of June). Thousands of tagged spawning horseshoe crabs have been observed by researchers over the past 10 years, and animals with 6–10-year-old tags have been recaptured (Swan 2005; Mattei et al. 2010; M.A.B. and J.H.M., unpublished data).

Previous studies have found that increased water temperatures or other stressors such as handling can increase mortality rates in some invertebrates (Kruse et al. 1994); increased water temperatures in this study had no effect on mortality of tagged horseshoe crabs. The water temperature in the raceways reached 26.2°C; even with this spike in water temperature, there was no concurrent increase in mortality of tagged crabs. This is unsurprising given that many horseshoe crabs are found stranded on the beach during low tide in the hot sun and are able to survive until the tide returns to submerge them (M.A.B. and J.H.M., personal observation).

In conclusion, no adult female horseshoe crabs died during a 44-d trial after being marked with a USFWS disk tag. Our results provide evidence that the tagging of adult horseshoe crabs is unlikely to cause an increase in mortality within a breeding season. Horseshoe crabs are usually tagged during May and June, and tagging activity should not lessen their ability to survive through the 30–45-d spawning season in Connecticut.

ACKNOWLEDGMENTS

We thank Chris Brown, Jose Pereira, and Sheila Stiles (National Oceanic and Atmospheric Administration, Northeast Marine Fisheries Science Center, Milford Laboratory) for allowing us to use their raceways and providing help with the setup of the raceways. We also thank Penny Howell (Connecticut Department of Environmental Protection, Marine Fisheries Unit) for providing the fish and squid used as feed. Lastly, we thank Sheila Eyler (USFWS, Maryland Fishery Resources Office) for providing the tags used in this study. The manuscript was greatly improved by the efforts of three anonymous reviewers.

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