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打鬥勝敗經驗效應展現之差異性、一致性與母體效應(第3年)

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中文摘要：近年來有非常多的研究顯示不同個體會展現不同的行為反應，但是相同個體的行為往往具有一致性。例如，有些個體持續的在不同的情境之下（例如覓食、交配等）展現出較其他個體來的高的攻擊性。有些理論提出個體之狀態（包含形態、生理、環境等）會因為影響個體行為之利益與代價而造成不同個體展現出不同的行為傾向。除了自身狀態之外，許多研究證據亦顯示母體之經歷也會影響子代之行為傾向與反應程度，這些影響可能使個體之行為反應更適合其環境狀況。此外，過去的相關經歷因為可以提供個體有用的資訊，而影響其再次遇到類似狀況時的行為決策。對動物打鬥而言，先前的落敗經驗往往使個體於遇到下一個對手時表現出較退縮的行為而再次被擊退（敗者效應）；相反的，獲勝的經驗會使個體變的較兇猛而再次獲勝（勝者效應）。勝、敗者效應之產生可能是因為打鬥經驗影響個體對自身打鬥能力與打鬥代價的評估，進而影響到後續的打鬥行為與獲勝機率。雖然勝敗者效应在許多物種都被觀察到，然而不同個體對勝敗經驗之反應程度是否具有一致性的差異卻未曾被檢視過。本計畫使用北美紅樹林鱗魚以兩個子題來探討相關議題。子題一探討不同個體對勝敗經驗之反應程度是否具有一致性的差異，以及這些差異與一致性是否與個體之狀態（競爭能力與荷爾蒙濃度）相關。子題二探討親代（母方）之勝敗經驗是否會影響子代之行為傾向與反應程度，以及親代之行為與生理狀態是否能預測其子代之行為與生理狀態。子題一之研究結果顯示（1）此魚之攻擊性（對自己鏡像之攻擊頻率）、睪固酮、皮質醇等皆具有一致性，一個月前與後的測量值呈現顯著正相關，（2）攻擊性的一致性高於荷爾蒙的一致性，建議荷爾蒙並非維持攻擊傾向一致性的主要生理機制，以及，（3）個體對獲勝或落敗經驗之反應並不具有一致性。子題二之研究結果顯示（1）親代之勝敗經驗會影響子代之攻擊性以及子代對勝敗經驗的反應：具落敗經驗親代所產下之子代具有較高的攻擊性，而具獲勝經驗之親代所產下之子代對落敗經驗有較高的反應程度（亦即展現出較高的敗者效應），（2）攻擊性之一致性受到經驗之影響，獲得落敗經驗之親代個體之一致性較低，（3）子代與親代之攻擊性、對經驗之反應程度以及賀蒙濃度皆未呈現正相關（親代與子代之睪固酮濃度甚至呈現顯著負相關），此外（4）品系對親代與子代之攻擊性與賀蒙濃度的影響不具有一致性。整體而言這些結果顯示，不同個體紅樹林鱗魚之攻擊性具一致性之差異，但是相關之生理機制並不明確，需要進一步探討。對此魚而言，親代的經歷對子代的行為有重要的影響，但是此魚之攻擊性與荷爾蒙之遺傳力可能不高。

中文關鍵詞：行為一致性、勝敗者效應、北美紅樹林鱗魚、反應程度、狀態、母體效應

英文摘要：A large amount of studies have shown that individuals tend to behave consistently but differently over time and across contexts. For instance, some individuals tend to exhibit higher levels of aggression in different contexts (e.g., foraging, mating) than the other individuals. State-dependent personality models suggest that an individual's state (morphology, physiology, environment, etc.) affects

the balance between the costs and benefits of different behaviors and prompts it to behave consistently in certain ways. In addition to an individual's state, maternal experience is also important in shaping an individual's behavioral tendency and responsiveness. Maternal influences could help the offspring to better cope with the environment. Individuals that have won or lost a fight recently are more likely to win and lose again, respectively (winner/loser effect). Different individuals may differ in their propensity to modify behavior after winning/losing experience. Despite the prevalence of winner and loser effects in the animal kingdom, differences and consistency in individual response to winning or losing experiences have not been investigated. Using mangrove killifish, *Kryptolebias marmoratus*, this project investigated (Study 1) whether different individuals of the fish show different but consistent responsiveness to winning or losing experiences, and if so, whether the responsiveness/consistency is associated with the individual's behavioral tendency and intrinsic state, and (Study 2) the importance of maternal experience to offspring's behavioral tendency and responsiveness as well as the importance of maternal physiology and behavior in predicting the offspring's physiology and behavior. The results of Study 1 showed that (1) the attack rate and the levels of testosterone and cortisol are highly consistent in the fish, (2) the consistency in the attack rate was higher than the consistency in the hormone levels, indicating that the levels of these hormones are not the physiological mechanisms that promoted the consistency in the attack rates, and (3) individuals of the fish did not appear to respond consistently to winning or losing experiences. The results of Study 2 showed that (1) parents' contest experiences significantly affected offspring's aggressiveness and responses to contest experiences: the offspring of parents with losing experiences attacked their mirror images at higher rates and showed greater loser effects, (2) parent fish that received losing experiences had lower consistency in their aggressiveness, (3) parent's and offspring's levels of aggressiveness and hormones were not positive correlated, (4) lineage did not have consistent influences on parent's and offspring's levels of aggressiveness or hormones. Overall, these results showed the fish's aggressiveness to be consistent over time, but the physiological mechanisms important to the consistency remain unclear. Although parents' contest experiences had important influences on the offspring's behaviors, the heritability of the fish's

behavior and hormone levels might not be very high.

英文關鍵詞：behavioral consistency, winner-loser effect, *Kryptolebias marmoratus*, responsiveness, state, maternal effect

Contents

	Page number
Chinese Abstract and Keywords.....	II
English Abstract and Keywords.....	III
Introduction.....	1
Materials and Methods.....	5
Results.....	10
Discussion.....	17
References.....	20

中文摘要

近年來有非常多的研究顯示不同個體會展現不同的行為反應，但是相同個體的行為往往具有一致性。例如，有些個體持續的在不同的情境之下（例如覓食、交配等）展現出較其他個體來的高的攻擊性。有些理論提出個體之狀態（包含形態、生理、環境等）會因為影響個體行為之利益與代價而造成不同個體展現出不同的行為傾向。除了自身狀態之外，許多研究證據亦顯示母體之經歷也會影響子代之行為傾向與反應程度，這些影響可能使個體之行為反應更適合其環境狀況。此外，過去的相關經歷因為可以提供個體有用的資訊，而影響其再次遇到類似狀況時的行為決策。對動物打鬥而言，先前的落敗經驗往往使個體於遇到下一個對手時表現出較退縮的行為而再次被擊退（敗者效應）；相反的，獲勝的經驗會使個體變的較兇猛而再次獲勝（勝者效應）。勝、敗者效應之產生可能是因為打鬥經驗影響個體對自身打鬥能力與打鬥代價的評估，進而影響到後續的打鬥行為與獲勝機率。雖然勝敗者效应在許多物種都被觀察到，然而不同個體對勝敗經驗之反應程度是否具有一致性的差異卻未曾被檢視過。本計畫使用北美紅樹林鱒魚以兩個子題來探討相關議題。子題一探討不同個體對勝敗經驗之反應程度是否具有一致性的差異，以及這些差異與一致性是否與個體之狀態（競爭能力與荷爾蒙濃度）相關。子題二探討親代（母方）之勝敗經驗是否會影響子代之行為傾向與反應程度，以及親代之行為與生理狀態是否能預測其子代之行為與生理狀態。子題一之研究結果顯示（1）此魚之攻擊性（對自己鏡像之攻擊頻率）、睪固酮、皮質醇等皆具有一致性，一個月前與後的測量值呈現顯著正相關，（2）攻擊性的一致性高於荷爾蒙的一致性，建議荷爾蒙並非維持攻擊傾向一致性的主要生理機制，以及，（3）個體對獲勝或落敗經驗之反應並不具有一致性。子題二之研究結果顯示（1）親代之勝敗經驗會影響子代之攻擊性以及子代對勝敗經驗的反應：具落敗經驗親代所產下之子代具有較高的攻擊性，而具獲勝經驗之親代所產下之子代對落敗經驗有較高的反應程度（亦即展現出較高的敗者效應），（2）攻擊性之一致性受到經驗之影響，獲得落敗經驗之親代個體之一致性較低，（3）子代與親代之攻擊性、對經驗之反應程度以及賀蒙濃度皆未呈現正相關（親代與子代之睪固酮濃度甚至呈現顯著負相關），此外（4）品系對親代與子代之攻擊性與賀蒙濃度的影響不具有一致性。整體而言這些結果顯示，不同個體紅樹林鱒魚之攻擊性具一致性之差異，但是相關之生理機制並不明確，需要進一步探討。對此魚而言，親代的經歷對子代的行為有重要的影響，但是此魚之攻擊性與荷爾蒙之遺傳力可能不高。

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English Abstract

A large amount of studies have shown that individuals tend to behave consistently but differently over time and across contexts. For instance, some individuals tend to exhibit higher levels of aggression in different contexts (e.g., foraging, mating) than the other individuals. State-dependent personality models suggest that an individual's state (morphology, physiology, environment, etc.) affects the balance between the costs and benefits of different behaviors and prompts it to behave consistently in certain ways. In addition to an individual's state, maternal experience is also important in shaping an individual's behavioral tendency and responsiveness. Maternal influences could help the offspring to better cope with the environment. Individuals that have won or lost a fight recently are more likely to win and lose again, respectively (winner/loser effect). Different individuals may differ in their propensity to modify behavior after winning/losing experience. Despite the prevalence of winner and loser effects in the animal kingdom, differences and consistency in individual response to winning or losing experiences have not been investigated. Using mangrove killifish, *Kryptolebias marmoratus*, this project investigated (Study 1) whether different individuals of the fish show different but consistent responsiveness to winning or losing experiences, and if so, whether the responsiveness/consistency is associated with the individual's behavioral tendency and intrinsic state, and (Study 2) the importance of maternal experience to offspring's behavioral tendency and responsiveness as well as the importance of maternal physiology and behavior in predicting the offspring's physiology and behavior. The results of Study 1 showed that (1) the attack rate and the levels of testosterone and cortisol are highly consistent in the fish, (2) the consistency in the attack rate was higher than the consistency in the hormone levels, indicating that the levels of these hormones are not the physiological mechanisms that promoted the consistency in the attack rates, and (3) individuals of the fish did not appear to respond consistently to winning or losing experiences. The results of Study 2 showed that (1) parents' contest experiences significantly affected offspring's aggressiveness and responses to contest experiences: the offspring of parents with losing experiences attacked their mirror images at higher rates and showed greater loser effects, (2) parent fish that received losing experiences had lower consistency in their aggressiveness, (3) parent's and offspring's levels of aggressiveness and hormones were not positive correlated, (4) lineage did not have consistent influences on parent's and offspring's levels of aggressiveness or hormones. Overall, these results showed the fish's aggressiveness to be consistent over time, but the physiological mechanisms important to the consistency remain unclear. Although parents' contest experiences had important influences on the offspring's behaviors, the heritability of the fish's behavior and hormone levels might not be very high.

Keywords: behavioral consistency, winner-loser effect, *Kryptolebias marmoratus*, responsiveness, state, maternal effect

Introduction

That previous winning and/or losing experiences affect subsequent contest decisions has been detected in individuals of all different taxa (winner/loser effect; Hsu et al. 2006). There has been no investigation, however, into whether different individuals of the same species differ in their responsiveness to wins or losses or whether the same individual responds consistently to the same experiences. Nor has the importance of maternal experience to an individual's responses to wins and losses been investigated. This project used a hermaphroditic fish, *Kryptolebias marmoratus*, as the study animal to start addressing these issues.

Animals often fight with each other to compete for resources that are important to their survival and reproduction. Fighting can, however, be costly in terms of expenditure of time and energy (Neat et al. 1998) and risks of both physical injury (Austad 1983; Neat et al. 1998) and predation (Brick 1999). Individuals are therefore expected to evaluate the potential costs and benefits associated with a contest and adjust their behavior accordingly (Maynard Smith & Price 1973; Maynard Smith 1974). Empirical studies have provided evidence that contest behavior and outcome are indeed affected by the costs and benefits perceived by contestants (Austad 1983; Dugatkin & Ohlsen 1990; Turner 1994).

Contest decisions are also influenced by the experience of previous victories and defeats (see Hsu et al. 2006 for a review). Individuals with a recent winning experience have a tendency to behave more aggressively and have a higher probability of escalating a contest (winner effect), while individuals with a recent losing experience behave submissively and retreat immediately when challenged (loser effect). Winner and loser effects probably arise because fight outcomes change an individual's perception of its fighting ability (Whitehouse 1997; Mesterton-Gibbons 1999) and consequently its estimation of the costs of taking part in later contests. Individuals with a winning experience perceive themselves to have good fighting abilities, expect low costs and are more willing to participate in future fights while individuals with a losing experience perceive and behave in the opposite way. Winner and loser effects are reported for animals of all taxa including insects (Adamo & Hoy 1995), arachnids (Hoefler 2002), crustaceans (Bergman et al. 2003), fish (Bakker et al. 1989; Chase et al. 1994), reptiles (Schuett 1997), birds (Martin et al. 1997) and mammals (Huhman et al. 2003). Because winner and loser effects are common phenomena in animal contests, many studies have been devoted to measuring their presence and persistence in different species and/or identifying physiological mechanisms associated with these effects (Li et al. 2014).

Winner-loser effects are usually regarded as species-specific characteristics which some species display but others do not. Recent studies in my laboratory, however, have revealed that individuals of the same species can differ in the extent of their response to winning and/or losing experiences (Lan & Hsu 2011; Earley et al. 2013). In the mangrove killifish, *Kryptolebias marmoratus*, the detectability of winner-loser effects depends on the hormonal state and the outcomes of fights experienced one month previously. The effects are more detectable in individuals (1) with lower levels of cortisol, testosterone and 11-ketotestosterone than others (Earley et al. 2013)

and (2) that were given a forced losing experience one month previously than in those that received a forced winning experience (Lan & Hsu 2011). These results suggest that the propensity to alter contest decisions in the light of the new information from recent wins and/or losses could be modulated by intrinsic and extrinsic factors. An individual's responsiveness to winning and/or losing experiences may, therefore, be plastic and susceptible to the influence of intrinsic and extrinsic factors. The responsiveness to winning and/or losing experiences may also differ between different individuals and reflect between-individual differences in the estimated payoffs of utilizing the information.

In recent years a great deal of research effort has been devoted to examining and understanding between-individual differences and within-individual consistency in behavioral tendencies and responsiveness. Accumulating results from empirical studies indicate that different individuals tend to behave differently but consistently over time and exhibit different 'personality' types (Stamps & Groothuis 2010). For example, if an individual behaves more aggressively than others at time A, it is also likely to behave more aggressively than others at time B (and is deemed to have an "aggressive" personality). Various theoretical models have been proposed to address the adaptive value of personality differences (reviewed in Dingemanse & Wolf 2010; Wolf et al. 2011). State-dependent personality models, for instance, suggest that personality is an adaptive outcome of among-individual differences in state (morphology, physiology, environment, etc.): individuals differ in state, which affects the balance between the costs and benefits of their actions and consequently their behavioral tendencies (see Sih et al. 2015 for a review). Individuals possessing low residual reproductive value, for example, should consistently be more bold and aggressive than individuals with high residual reproductive value. Consistency in behaviors could also reflect positive feedback mechanisms between state and behavior. For example, stronger individuals are more aggressive, which gives them access to better food sources, which causes them to become even stronger and behave more aggressively.

Although individuals tend to behave consistently, this does not exclude plasticity in their behavior (Dingemanse et al. 2010). Individuals can adjust their behavior when facing different environmental conditions (plasticity) but remain consistently different from each other (personality) (Mathot et al. 2012). And the propensity to modify behavior in response to environmental changes (i.e., plasticity or responsiveness) could vary between individuals with different behavioral tendencies (Stamps 2016). In barnacle geese (*Branta leucopsis*), for instance, individuals behave consistently in terms of boldness, and bolder individuals have a lower tendency to modify feeding behavior based on social information (Kурvers et al. 2010).

Being responsive to new information would allow an individual to behave in a more adaptive way in an unstable environment (Mery & Burns 2010). However, it is not without costs. Monitoring internal and external environments (Niven & Laughlin 2008) and learning and maintaining accurate information (Dukas 1999; Burns et al. 2011), for instance, are costly processes. Flies (*Drosophila melanogaster*) that were selected for improved learning ability ('High-learning lines') produced fewer eggs after repeated associative learning training, whereas the "Low-learning lines" that had not been selected in this way did not (Mery & Kawecki 2004). Flies that were induced to form

long-term memory did not survive deprivation of food and water as long as other flies (Mery & Kawecki 2005). Because there is always uncertainty in how the environment might change, there is a risk that acquired information might not be applicable to the new environment, which could introduce additional costs to being responsive. Different individuals could differ in the expected benefits and costs of modifying behavior in the light of new information, leading to differences in their responsiveness.

The same aspects of an individual's state (morphology, physiology, environment, etc.) that affect the balance between the costs and benefits of its behavioral actions (Sih et al. 2015) could also affect the balance between the costs and benefits of being responsive (Dingemanse & Wolf 2013). The consistency in an individual's state would then promote a consistency in individual responsiveness. For instance, how far an individual should move in response to a change in resource abundance may depend on that individual's energetic state (Dingemanse & Wolf 2013). If individuals with lower levels of energy storage tend to be less responsive to a decrease in local resource abundance and have lower feeding rates in return, they would tend to remain in a low energetic state (consistency in state) and continue to display low responsiveness to a change in local resource abundance (consistency in responsiveness).

Maternal conditions can also have an important influence on the physiology and behavioral performance of the offspring (Mousseau & Fox 1998; Green 2008; Fougerey 2013). Maternal experience with social or asocial environments can affect the development, physiology and behavior of the offspring in ways that enable the offspring to be better suited to the environments. In farmed Atlantic salmon (*Salmo salar*), the offspring of females that were injected with higher levels of cortisol (a stress hormone) were less active (Espmark et al. 2008). Eggs produced by female three-spined sticklebacks (*Gasterosteus aculeatus*) exposed to the threat of predation were larger, had higher cortisol content and hatched into juveniles that exhibited tighter shoaling behavior (an antipredator defense) than those produced by females in the control group (Giesing et al. 2011). And the offspring of gravid female crickets (*Gryllus pennsylvanicus*) that have been exposed to a predatory wolf spider *Hogna helluo* exhibit greater antipredator immobility in response to the spiders' chemical cues than do offspring of non-exposed females (Storm & Lima 2010). The offspring from female predatory mites (*Phytoseiulus persimilis*) that were exposed to the intraguild predator (*Amblyseius andersoni*) during egg production showed a greater response to the intraguild predator's cues (Seiter & Schausberger 2015).

As previously described, the winning and losing outcomes of recent fights are assumed to provide individuals with information about how their ability to compete compares with the abilities of others in the population (Whitehouse 1997). Individuals modify their contest behaviors based on these outcomes (winner-loser effects; Hsu et al. 2006) so that their contest decisions become more appropriate in the light of the newly acquired information. Despite the prevalence of winner and loser effects in the animal kingdom and the vast interest in behavioral responsiveness and consistency in recent behavioral literature, whether and how (1) different individuals might respond differently and/or consistently to winning or losing experiences, and (2) a female's experience of wins and losses might influence the behavior of its offspring have not been investigated. Building

on our recent findings that the detectability of winner-loser effects in the self-fertilizing, hermaphroditic mangrove killifish, *Kryptolebias marmoratus*, depends on intrinsic and extrinsic factors (Lan & Hsu 2011; Earley et al. 2013), this project addressed these questions using the fish as the study animal.

Study 1: Whether different individuals show different but consistent responsiveness to winning and losing experiences, and, if so, whether the responsiveness and consistency is associated with the individual's behavioral tendency (in terms of aggressiveness) and intrinsic state (fighting ability and hormone profiles). The results from our previous studies on the fish's winner-loser effects showed that these effects were more detectable in individuals (1) that have or perceive themselves to have a worse fighting ability (Lan & Hsu 2011) and (2) that have hormonal profiles corresponding to lower aggressiveness and worse fighting abilities (Earley et al. 2013). One possible explanation for these results is that individuals with worse competitive ability or lower aggressiveness have inferior body conditions (worse nutritional state: slow recovery from injuries; lower energy storage: lower capability to replenish the energy consumed in contest interactions). Although resources being competed for may be highly valuable, the cost of losing is also very high to these individuals, which prompts them to be more attentive to the newly acquired fighting ability related information. Based on the results of the previous studies, we expected the fish's responsiveness to contest experiences to be negatively associated with its fighting ability, aggressiveness and levels of endogenous steroid hormones.

Study 2: (I) The importance of maternal experience to offspring's aggressiveness and responsiveness, and (II) the importance of maternal hormone profiles, aggressiveness and responsiveness to the corresponding traits in the offspring. Studies have shown that competitive ability, aggressiveness and dominance ability are heritable (Nol et al. 1996; Cunningham et al. 2013; Weiß & Foerster 2013). An individual's winning-losing experiences could, therefore, provide sampling information about the relative competitive ability not only for the individual but also for its offspring. If maternal experience in the fish has an adaptive effect on the offspring's behavior, the offspring of individuals with winning/losing experiences should behave more/less aggressively than the offspring of control individuals (with no contest experiences). And, because the responsiveness to contest experiences is expected to be negatively associated with aggressiveness, the offspring of individuals with winning/losing experiences are expected to be less/more sensitive to the influence of contest experiences. This study also examined whether the maternal hormone profiles, aggressiveness and responsive ness predict the corresponding traits in the offspring.

Materials and Methods

I. The study species

The mangrove killifish *Kryptolebias marmoratus*, is an internally self-fertilizing androdioecious fish (Taylor 2012) and inhabits mangrove areas ranging from south-eastern Brazil, Venezuela, throughout much of the Caribbean, the Bahamas, Yucatan and southern Florida (Harrington & Kallman 1968). Natural populations consist mainly of isogenic, hermaphrodites with very low incidence (< 1%) of males, although an outcrossing heterozygous population with 20% males was discovered in Twin Cays, Belize (Mackiewicz et al. 2006). Adult hermaphroditic descendants of 7 isogenic lineages of *K. marmoratus* originally collected by Dr. D. Scott Taylor and Dr. Ryan Earley from various locations (BP9: Big Pine Key, Florida, USA 2010; DAN2K: Dangria, Belize, collected in 2000; DC1: Dove Creek, Tavernier, Florida, USA; HON9: Utila, Honduras, 1996; RHL: San Salvador, Bahamas, 1997; SLC8E: St. Lucie County, FL, USA, 1995; VOL: Volusia County, Florida, USA, 1995) were used for this study. Fish were kept individually in 13 x 13 x 9 cm translucent polypropylene plastic containers (maintenance container). Every container was filled with 600 ml of approximately 25 ppt synthetic sea water (Instant Ocean[®] powder) and labelled with a unique code for individual identification. Water in all containers was replaced monthly. Fish were maintained at 25°C on a 14:10 h light:dark cycle and fed newly hatched brine shrimp (*Artemia*) nauplii daily.

II. Experimental design

Study 1: Whether different individuals show different but consistent responsiveness to winning and losing experiences, and, if so, whether the responsiveness and consistency is associated with the individual's behavioral tendency (in terms of aggressiveness) and intrinsic state (fighting ability and hormone profiles).

For this study, we examined whether different individuals display different but consistent tendencies to alter their aggressiveness after being exposing to a winning or losing experience, and whether the tendency is related to an individual's personality (in terms of aggressiveness), fighting ability or endogenous levels of hormones. We used a 3 (experience treatment: winning (W), losing (L), or no-contest experience (N)) × 2 (fighting ability: better or worse) factorial design for these objectives (Table 1). In summary, the same individual received the same type of experience twice (4 weeks apart). We then examined whether the behavioral changes following the two were similar.

The procedures for study 1 are provided in Table 2. Approximately 4 ~ 6 weeks prior to Day1, contests were staged at 9:00 between size-matched (difference in body length ≤ 1 mm) individuals to divide them into two “fighting ability” groups: with better (winners of the contests) or worse (losers of the contests) fighting ability. The procedures for staging contests are described in detail

		<i>Fighting ability</i>	
		Better	Worse
<i>Experience</i>	Winning	n = 35	n = 35
	Losing	n = 35	n = 35
	No-contest (control)	n = 35	n = 35

Table 1. The experimental design for Study 1.

below in ‘**III. General Procedures: 1. Staging contests**’. Fish were replaced in their maintenance containers after the contest and fed newly hatched brine shrimp nauplii. Winners/losers of the contests were deemed to have “better”/”worse” fighting ability. Fish with “better” or “worse” fighting ability were randomly assigned to one of the 3 experience (W, L or N) treatments.

		1 st responsiveness measure			2 nd responsiveness measure		
	4-6 W prior to Day1	Day1	Day2	Day3	Day29	Day30	Day31
Morning	Size-matched Contest	Hormone Sample #1	Pre-experience aggressiveness #1	Post-experience aggressiveness #1	Hormone Sample #2	Pre-experience aggressiveness #2	Post-experience aggressiveness #2
Afternoon			Experience #1			Experience #2	

Table 2. The time schedule for Study 1’s experimental procedures.

On Day1, water samples were collected from the study fish at 9:00 for their first hormone measurement. (See ‘**III. General procedure: 2. Hormone collection, extraction and assay**’). Fish were replaced in their maintenance containers after the procedures and fed newly hatched brine shrimp nauplii.

On Day2, at 10:00, the study fish were prepared for their first pre-experience aggressiveness test (see ‘**III. General procedure: 3. Aggressiveness test**’). Upon the completion of the test, the fish were replaced in their maintenance containers and fed newly hatched brine shrimp nauplii. On the same day at 15:00, the fish were prepared to receive their predesigned contest experience for the first time (see ‘**III. General procedure: 4. Providing a winning, losing or no-contest experience**’). Upon the completion of the experience training, the fish were replaced in their maintenance containers.

On Day 3, at 10:00, the study fish were prepared for their first post-experience aggressiveness test (see ‘**III. General procedure: 3. Aggressiveness test**’). Upon the completion of the test, the fish were replaced in their maintenance containers and fed newly hatched brine shrimp nauplii.

On Days 29, 30 and 31, we repeated the procedures for Days1, 2, and 3, respectively for the 2nd hormone measurement, pre-experience aggressiveness test, contest-experience training and post-experience test.

Study 2: (I) The importance of maternal experience to offspring’s aggressiveness and responsiveness, and (II) the importance of material hormone profiles, aggressiveness and responsiveness to the corresponding traits in the offspring’s.

This study used a 3 (parent’s experience: 3 W, 3 L or 3 N) × 3 (offspring’s experience: 1 W, 1 L or 1 N) factorial design (Table 3) to examine whether the offspring’s behavioral tendency and responsiveness to winning and losing experiences were dependent on the parent’s contest history. The parents and offspring in this study were given the same experience 2 times to enforce the effects (Earley et al. 2013; Li et al. 2014). The parent’s and the offspring’s hormone profiles, aggressiveness and responsiveness to contest experience were measured to evaluate the ability of

the parental traits to predict the offspring's traits. One offspring (the first offspring that survived to 8-month old) from each parent was used for the study. As shown in Table 3, although we used 56 parents for each of the 9 treatments (a total of 504 parents), some of these parents did not produce viable offspring for follow-up experimental procedures. The final sample sizes for these treatments were between 28 to 35 (total sample size = 288).

		<i>Offspring's experience</i>		
		<i>2W</i>	<i>2L</i>	<i>2N</i>
<i>Parent's experience</i>	<i>2W</i>	n = 56 (32)	n = 56 (32)	n = 56 (32)
	<i>2L</i>	n = 56 (35)	n = 56 (35)	n = 56 (34)
	<i>2N</i>	n = 56 (28)	n = 56 (30)	n = 56 (30)

Table 3. The experimental design for Study 2. The numbers in the parentheses showed how many of the 56 parents actually produced viable offspring for the experimental procedures.

Fish that were between 8 and 15 months of age are used as the parent fish of this study. The parent fish are randomly assigned to one of the 9 parent-offspring experience treatments.

The procedures for Study 2 are summarized in Table 4. Approximately 4 ~ 6 weeks prior to Day1, contests were staged at 9:00 between size-matched (difference in body length ≤ 1 mm) individuals to divide them into two "fighting ability" groups: with better (winners of the contests) or worse (losers of the contests) fighting ability. The procedures for staging contests are described in detail

Parent	Parent/Offspring				
4 W prior to Day1	Day1	Day3	Day4	Day5	Day6
Size-matched Contest	Hormone sample	Pre-experience aggressiveness	1 st Experience	2 nd Experience	Post-experience aggressiveness

Table 4. The time schedule for Study 2's experimental procedures.

below in 'III. General Procedures: 1. Staging contests'. Fish were replaced in their maintenance containers after the contest and fed newly hatched brine shrimp nauplii. Winners/losers of the contests were deemed to have "better"/"worse" fighting ability. Fish with "better" or "worse" fighting ability were randomly assigned to one of the 3 experience (W, L or N) treatments.

On Day1, water samples were collected from the parent fish at 9:00 (See 'III. General procedure: 2. Hormone collection, extraction and assay'). After the procedures, we measured the fish's body length and weight. Afterwards, fish were replaced in their maintenance containers procedures and fed newly hatched brine shrimp nauplii.

No experimental procedures were conducted on Day2 to allow the fish one day to recover from the hormone measurement procedures.

On Day3, the parent individuals were subjected to the aggressiveness test (pre-experience aggressiveness) (see 'III. General procedure: 3. Aggressiveness test'). Upon the completion of the test, the fish were replaced in their maintenance containers and fed newly hatched brine shrimp nauplii.

On Day4, the fish were subjected to the predesigned contest experience for the first time (see 'III. General procedure: 4. Providing a winning, losing or no-contest experience'). Upon the completion of the experience training, the fish were replaced in their maintenance containers. On Day5, the fish received their predesigned contest experience for the 2nd time to reinforce the effect of the experience. Fish were replaced in their maintenance containers after procedures and fed newly hatched brine shrimp nauplii.

On Day6, the fish were subjected to their post-experience aggressiveness test (see ‘**III. General procedure: 3. Aggressiveness test**’). Upon the completion of the test, the fish were replaced in their maintenance containers and fed newly hatched brine shrimp nauplii.

Between Day7 to Day35 (4-week period), the first 30 eggs laid by a parent fish were collected and placed in individual containers (120 ml container containing 50 ml clean 25 ppt salt water, 1 egg/container). For each of the parents, the first offspring that survived to 8 months of age was designated as its offspring to be used for the study. The offspring individuals were subject to the same experimental procedures as the parent individuals: Day1-hormone collection, Day3-aggressiveness test (pre-experience aggressiveness), Day4- 1st contest experience, Day5- 2nd contest experience, Day6- aggressiveness test (post-experience aggressiveness).

III. General Procedures

1. Staging contests:

Contests were staged between two individuals of a designated pair to divide experimental fish into those with better fighting ability (contest winners) and those with worse fighting ability (contest losers). To identify individuals, the non-vascularized, outer margins of the fish’s caudal fins were clipped without removing any tissue 24 hours before they were used for experiments. The two fish were placed one in each compartment, randomly selected, of a standard aquarium (12 × 16 × 20 cm³, containing water 12 cm deep and 2 cm of gravel) separated by a black partition. The two contestants were allowed to acclimatize in the standard aquarium until 09:00 next morning. A contest began when the partition was lifted. The contestant that first chased/attacked its opponent for 5 minutes without retaliation was the winner and its opponent the loser.

2. Hormone collection, extraction and assay

Fish were placed in individual 600 ml glass beakers with 400 ml clean 25 ppt synthetic sea water (one fish/beaker) and left in the beakers for 1 hour. After 1 hour, the fish were removed from the beakers. Hormone was extracted from the water samples using C18 solid phase extraction columns (Lichrolut RP-18, 500 mg, 3.0 ml; Merck) fitted to a 24-port manifold. Columns were first primed with 2 consecutive washes with 2 ml HPLC grade methanol (MeOH) followed by 2 consecutive washes with 2 ml distilled water. Tubing was then fastened securely to the top of each column and placed into the water sample collected from the fish. The vacuum was engaged and the water sample passed through the tubing into the column. When the samples passed completely through the columns, salts were removed with 2 consecutive 2 ml washes of distilled water. Columns were frozen until further processing. Freeze storage of water samples and columns has been determined not to impact steroid concentrations (Ellis et al. 2004). Columns were thawed and purged with 2 x 2 ml washes of distilled water. Hormone was eluted from the columns into 12 x 75 mm (6 ml) borosilicate vials by 2 consecutive 2 ml washes with HPLC grade MeOH. The 4 ml of eluted solvent was evaporated at 40 °C (water bath) with a gentle stream of nitrogen (~0.7n/m²).

The resulting hormone pellet was re-suspended in 800 μ l of enzyme-immunoassay (EIA) buffer supplied with the kits (see below) and the samples stored at -20 °C until assay.

Cayman Chemicals Inc. EIA kits were used for all hormones (Cortisol and T), following the manufacturer's recommended procedures. Plates were read at 405nm on a BioTek microplate reader. Earley and Hsu (2008) have validated the Cayman Chemicals, Inc. kits for *K. marmoratus* water-extracted hormones by assessing parallelism of a serial dilution curve with the standard curve and quantitative recovery.

Data were presented as pg/sample. Because the distributions of the levels of testosterone and cortisol tended to be skewed, we ln transformed these measurements for statistical analyses.

3. Aggressiveness test

We used the procedures modified from Chang et al. (2012) for the test. Aggressiveness was quantified by a mirror test in a standard aquarium (12 \times 16 \times 20 cm, containing water 12 cm deep and 2 cm of gravel). The mirror was attached to one of the narrow sides of the tank (randomly assigned), with the fish acclimatized behind an opaque partition 11 cm from the mirror for 30 min. After the acclimatization period, the partition was removed and the fish was allowed to interact with its mirror image for 30 min. Afterwards, the partition was re-inserted to separate the fish and the mirror to terminate the test. We calculated the attack rate (#/min) from the number of attacks the fish delivers at its mirror image in 5 minutes after the first attack and used it to quantify aggressiveness. The difference in the attack rate (#/min) between the post- and pre-experience aggressiveness test were calculated as the fish's response to contest experiences:

$$\text{Change in attack rate} = (\text{post-experience attack rate}) - (\text{pre-experience attack rate})$$

4. Providing a winning, losing or no-contest experience

To ensure that all individuals receive their pre-designated losing (or winning) experience, they were fought against much larger (smaller) trainer fish (difference in SL > 2mm) that have won (lost) several fights against similar-sized opponents. The experience training were staged by placing an experimental fish in one of the two similar-sized compartments (randomly assigned) of a standard aquarium divided by an opaque partition and the larger (smaller) trainer fish in the other compartment. After 30-min acclimatization, the partition was removed to allow the fish to interact. A losing experience was completed when the experimental fish retreated from a display/attack by the larger trainer fish and quickly swam away. A winning experience was completed when the smaller trainer fish retreated from the experimental individual's display/attack and quickly swam away. Fish assigned to receive a no-contest experience were treated exactly as above, except that there was no opponent in the other compartment and was allowed to swim around for 2 min before the partition was replaced. When a fish was assigned to receive multiple winning or losing experiences (one experience training per day for multiple days), a new trainer fish was used every day to avoid possible complication of individual recognition.

Results

Study 1: Whether different individuals show different but consistent responsiveness to winning and losing experiences, and, if so, whether the responsiveness and consistency is associated with the individual's behavioral tendency (in terms of aggressiveness) and intrinsic state (fighting ability and hormone profiles).

The test fish were randomly assigned to the three experience treatments in Study 1 such that there was no significance in the body size (SL) of the fish assigned to the three treatments ($F_{2,209} = 0.4$, $p = 0.636$; mean \pm SE: W = 29.3 \pm 0.4, N = 29.9 \pm 0.4; L = 29.6 \pm 0.4 mm). The mirror-attack rates (#attacks/min) measured before and after the 1st set of experiences and before and after the 2nd set of experience are all highly correlated (Table 5). Individuals of the fish thus show within-individual consistency in aggressiveness in aggressiveness. The fish's cortisol and testosterone levels measured one month apart also highly correlated (Table 5). The consistency in aggressiveness appears to be higher than the consistency in hormone levels (Table 5).

No significant experience effects on attack rates were detected for either experience 1 ($F_{2,207} = 1.4$, $p = 0.260$; mean \pm SE: W = 1.43 \pm 0.57, N = 0.21 \pm 0.57; L = 0.37 \pm 0.57) or experience 2 ($F_{2,207} = 0.1$, $p = 0.888$; mean \pm SE: W = 1.61 \pm 0.67, N = 1.21 \pm 0.67; L = 1.61 \pm 0.67). The fish did not show consistent changes in the attack rate after receiving a winning ($r = -0.03$, $n = 70$, $p = 0.811$), a losing ($r = -0.21$, $n = 70$, $p = 0.823$), or no fighting ($r = -0.14$, $n = 70$, $p = 0.432$) experiences.

Table 5. Pair-wise correlations between the four mirror attack rates measurements (#attacks/min, Att) and between the two cortisol (Cort) and testosterone (T) measurements.

Variable 1	Variable 2	r (95% CI)	p-value
Pre-Exp1-Att	Post-Exp1-Att	0.70 (0.62-0.76)	<0.0001
Pre-Exp1-Att	Pre-Exp2-Att	0.55 (0.45-0.64)	<0.0001
Pre-Exp1-Att	Post-Exp2-Att	0.38 (0.26-0.49)	<0.0001
Pre-Exp2-Att	Post-Exp1-Att	0.74 (0.68-0.80)	<0.0001
Pre-Exp2-Att	Post-Exp2-Att	0.65 (0.57-0.72)	<0.0001
Post-Exp1-Att	Post-Exp2-Att	0.58 (0.49-0.66)	<0.0001
Pre-Exp1-Cort	Pre-Exp2-Cort	0.20 (0.06-0.32)	0.0042
Pre-Exp1-T	Pre-Exp2-T	0.22 (0.09-0.34)	0.0014

Individuals with better or worse fighting ability did not differ significantly in the cortisol levels measured before experience 1 ($t_{208} = 0.8$, $p = 0.402$; mean \pm SE: Better = 288.4 \pm 26.9, Worse = 320.3 \pm 26.9) or experience 2 ($t_{208} = 0.3$, $p = 0.776$; mean \pm SE: Better = 313 \pm 36.0, Worse = 298.8 \pm 36.0). They also did not differ significantly in the testosterone levels measure before experience 1 ($t_{208} = 0.8$, $p = 0.444$; mean \pm SE: Better = 368.3 \pm 18.1, Worse = 348.7 \pm 18.1) or experience 2 ($t_{208} = 0.6$, $p = 0.567$; mean \pm SE: Better = 384.3 \pm 23.8, Worse = 365.0 \pm 23.8).

Individuals with better or worse fighting ability, however, differed (marginally) significantly in the attack rate measured before ($t_{208} = 2.3$, $p = 0.021$; mean \pm SE: Better = 7.4 \pm 0.6, Worse = 5.5 \pm 0.6) and after ($t_{208} = 2.4$, $p = 0.020$; mean \pm SE: Better = 8.1 \pm 0.6, Worse = 6.1 \pm 0.6) experience 1. Individuals with better fighting ability attacked their mirror images at higher rates than the ones with worse fighting ability both before and after experience 1. Individuals with better fighting ability also had higher attack rates before ($t_{208} = 2.3$, $p = 0.024$; mean \pm SE: Better = 7.3 \pm 0.6, Worse

= 5.4 ± 0.6) but not after ($t_{208} = 0.6$, $p = 0.536$; mean \pm SE: Better = 8.1 ± 0.7 , Worse = 7.5 ± 0.7) experience 2 than the ones with worse fighting ability. They also did not differ significantly in the changes of the attack rate after experience 1 ($t_{208} = 0.1$, $p = 0.893$; mean \pm SE: Better = 0.71 ± 0.47 , Worse = 0.62 ± 0.47) or experience 2 ($t_{208} = 1.7$, $p = 0.084$; mean \pm SE: Better = 0.81 ± 0.54 , Worse = 2.14 ± 0.54). Generally speaking, individuals with better a fighting ability attacked their mirror images at higher rates than individuals with a worse fighting ability. However, there is no evidence for fighting ability to play an important role of an individual's responsive after contest experiences.

We next analyzed the importance of an individual's fighting ability and pre-experience aggressiveness, testosterone and cortisol to its responsiveness to a winning or losing experience. For individuals that received a winning experience, the change in the attack rate after experience 1 related negatively with pre-experience aggressiveness ($b = -0.27 \pm 0.04$, $F_{1,68} = 6.8$, $p = 0.011$) and positively with testosterone ($b = 0.0068 \pm 0.0029$, $F_{1,68} = 5.4$, $p = 0.023$) and cortisol ($b = 0.0065 \pm 0.0024$, $F_{1,68} = 7.4$, $p = 0.008$) but had no relationship with fighting ability ($F_{1,68} = 0.3$, $p = 0.608$). For individuals that received a winning experience, the change in the attack rate after experience 2 did not have significant relationship with pre-experience aggressiveness ($b = -0.14 \pm 0.08$, $F_{1,68} = 3.2$, $p = 0.077$), testosterone ($b = -0.0020 \pm 0.0023$, $F_{1,68} = 0.8$, $p = 0.386$), cortisol ($b = -0.0014 \pm 0.0018$, $F_{1,68} = 0.6$, $p = 0.446$) or fighting ability ($F_{1,68} = 0.4$, $p = 0.507$). For individuals that received a losing experience, the change in the attack rate after experience 1 related negatively with pre-experience aggressiveness ($b = -0.24 \pm 0.09$, $F_{1,68} = 8.1$, $p = 0.006$) but not pre-experience testosterone ($b = -0.0037 \pm 0.0029$, $F_{1,68} = 1.6$, $p = 0.205$) or cortisol ($b = 0.0004 \pm 0.0016$, $F_{1,68} = 0.1$, $p = 0.8210$) or fighting ability ($F_{1,68} = 0.0$, $p = 0.924$). For individuals that received a losing experience, the change in the attack rate after experience 2 also related negatively with pre-experience aggressiveness ($b = -0.24 \pm 0.11$, $F_{1,68} = 4.8$, $p = 0.032$) but not pre-experience testosterone ($b = -0.0009 \pm 0.0026$, $F_{1,68} = 0.1$, $p = 0.732$) or cortisol ($b = -0.0016 \pm 0.0022$, $F_{1,68} = 0.5$, $p = 0.501$) or fighting ability ($F_{1,68} = 1.7$, $p = 0.194$).

Study 2(I): The importance of maternal experience to offspring's aggressiveness and responsiveness

The influence of contest experiences on parent's aggressiveness:

In this study, the parent individuals showed strong loser effects and attacked their mirror image at significantly lower rates after losing experiences ($F_{2,501} = 33.4$, $p < 0.0001$; mean \pm SE: W = 1.15 ± 0.43 , N = 0.99 ± 0.43 ; L = -3.3 ± 0.43 ; Figure 1). For these parent individuals, pre- and post-experience attack rates were positively correlated ($r = 0.46$, 95% CI: 0.39-0.53, $n = 504$, $p < 0.0001$). When analyzed separately, the correlations were smaller for the ones that received losing experience treatments ($r = 0.35$, 95% CI: 0.21-0.47, $n = 168$, $p < 0.0001$) than for the ones that received winning ($r = 0.56$, 95% CI: 0.47-0.66, $n = 168$, $p < 0.0001$) or no fighting ($r = 0.58$, 95% CI: 0.47-0.67, $n = 168$, $p < 0.0001$) experience treatments (Figure 2). These results showed that losing experience lowered aggressiveness as well as the consistency in aggressiveness in the parent individuals.



Figure 1. The change (post-experience – pre-experience) in attack rates (#/min, mean \pm SE) of the parent individuals after experience treatments. Means labeled with different letters are significant different at $P < 0.05$ (Tukey multiple comparisons).

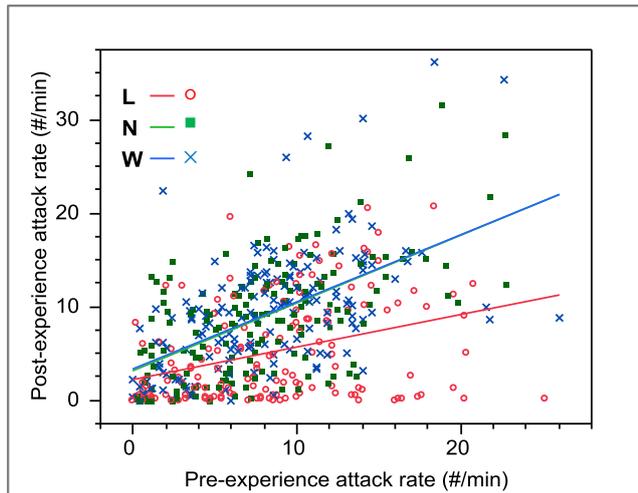


Figure 2. The relationship between pre- and post-experience attack rate (#/min, mean \pm SE) of parent individuals that received different experience treatments)

Parent individuals that had better fighting ability attacked mirror images at a higher rate before exposed to contest experiences ($t_{502} = 5.3$, $p < 0.001$; mean \pm SE: better = 9.23 ± 0.31 , worse = 6.90 ± 0.31). Parent’s fighting ability, however, did not affect the change in the attack rate after receiving contest experience ($p = 0.258$) or their responsiveness to contest experiences ($p = 0.142$) (Table 6).

Table 6. The importance of fighting ability (FA) and contest experience (Exp) on parent’s change in attack rate after receiving contest experiences.

	<i>F</i>	<i>dfs</i>	<i>p</i>
FA	1.3	1,498	0.258
Exp	33.6	2,498	<0.001
FA \times Exp	2.0	2,498	0.142

The influence of parent’s contest experience on the offspring’s aggressiveness and hormones:

Offspring of the parents that received different contest experiences differed significantly in their pre-experience attack rates ($F_{2,285} = 4.7$, $p = 0.008$; mean \pm SE: W = 11.8 ± 0.6 , N = 10.5 ± 0.6 ; L = 13.3 ± 0.6 ; Figure 3). The trend was for the offspring of the parents that were given the control experience (i.e., no contest experiences) to have lower attack rates than the offspring of the parents that received either losing or winning experiences (although only the mean of the offspring of the parents that were given losing was significantly higher) (Figure 3). Parent’s

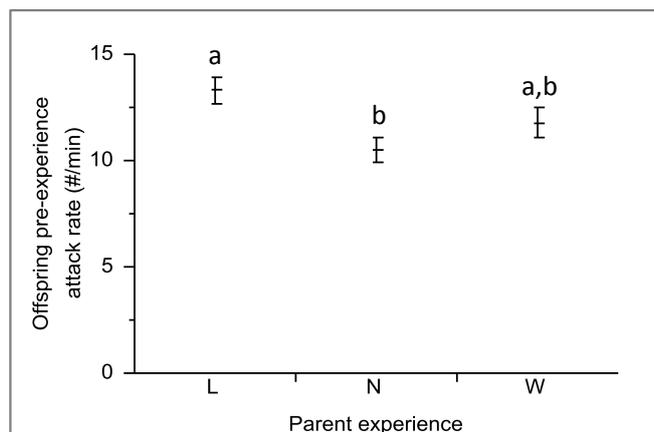


Figure 3. The offspring’s pre-experience attack rate (#/min, mean \pm SE) of parents that received different contest experiences. Means labeled with different letters are significant different at $P < 0.05$ (Tukey multiple comparisons).

contest experiences, however, did not have significant influence on the offspring's level of testosterone ($F_{2,285} = 0.9, p = 0.398$) or cortisol ($F_{2,285} = 1.2, p = 0.304$).

The importance of parent's contest experience on offspring's sensitivity to contest experiences:

Both the parent's and offspring's contest experience had significance influences on the offspring's responsiveness to contest experiences (general linear model; parent: $p = 0.001$; offspring: $p = 0.004$; Table 7).

Furthermore, parent's and offspring's contest experiences had insignificant interaction effects on the offspring's attack rate (parent×offspring: $p = 0.089$) (Table 6). Further analyses showed that the offspring significantly responded to contest experiences when the parents received

winning ($F_{2,279} = 7.1, p = 0.001$) experiences but not if the parent's received losing ($F_{2,279} = 0.1, p = 0.898$) or no contest ($F_{2,279} = 2.2, p = 0.113$) experiences (Figure 4).

Table 7. The joint influence of the parent's and the offspring's contest experiences on the offspring's attack rate.

	<i>F</i>	<i>dfs</i>	<i>p</i>
Parent	11.7	2,279	0.001
Offspring	6.7	2,279	0.004
Parent×Offspring	13.0	4,279	0.089

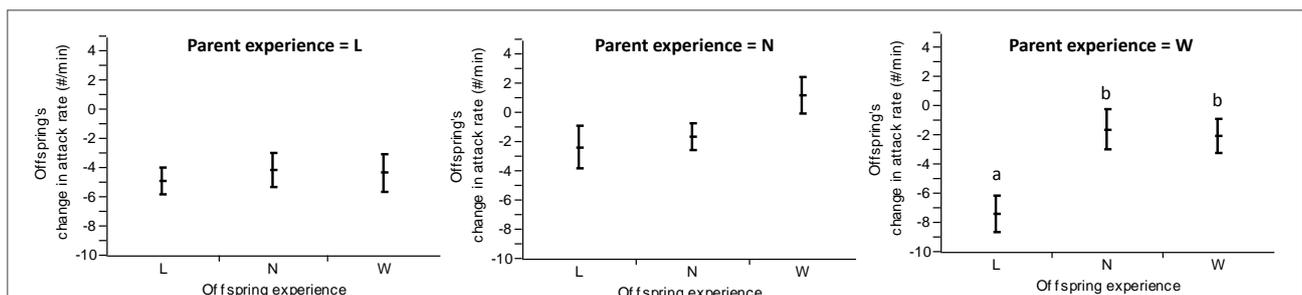


Figure 4. The change in attack rate ((#/min, mean \pm SE) after receiving different contest experiences of offspring who's parents were exposed to different contest experiences. Means labeled with different letters are significant different at $P < 0.05$ (Tukey multiple comparisons).

Study 2 (II) the importance of material hormone profiles, aggressiveness and responsiveness to the corresponding traits in the offspring's.

Importance of parent's aggressiveness and responsiveness to contest experiences to those of the offspring:

Parent's fighting ability did not have influence on the offspring's aggressiveness (i.e., offspring's pre-experience attack rate; $t_{286} = 0.1, p = 0.928$; mean \pm SE: better = 11.98 ± 0.52 , worse = 11.91 ± 0.53). Furthermore, parent's aggressiveness (pre-experience attack rate) did not predict offspring's aggressiveness (pre-experience attack rate) (general linear model, $b = 0.07\pm 0.07, F_{1,286} = 0.8, p = 0.371$). We used the offspring individuals that received the same experience treatments as their parents to examine whether the parent's responsiveness would predict offspring's responsiveness to the same contest experiences. The analyses showed that the parent's responsiveness did not have significant predictive power of the offspring's responsiveness (general

linear models; W: $b = -0.43 \pm 0.28$, $F_{1,30} = 2.3$, $p = 0.142$; N: $b = 0.03 \pm 0.16$, $F_{1,28} = 0.03$, $p = 0.865$; L: $b = -0.15 \pm 0.13$, $F_{1,33} = 1.3$, $p = 0.263$).

Importance of parent’s hormonal state to offspring’s hormonal state:

Because the distributions of parent’s and offspring’s levels of testosterone and cortisol were skewed, these measurements were ln transformed for statistical analyses. The parent’s levels of

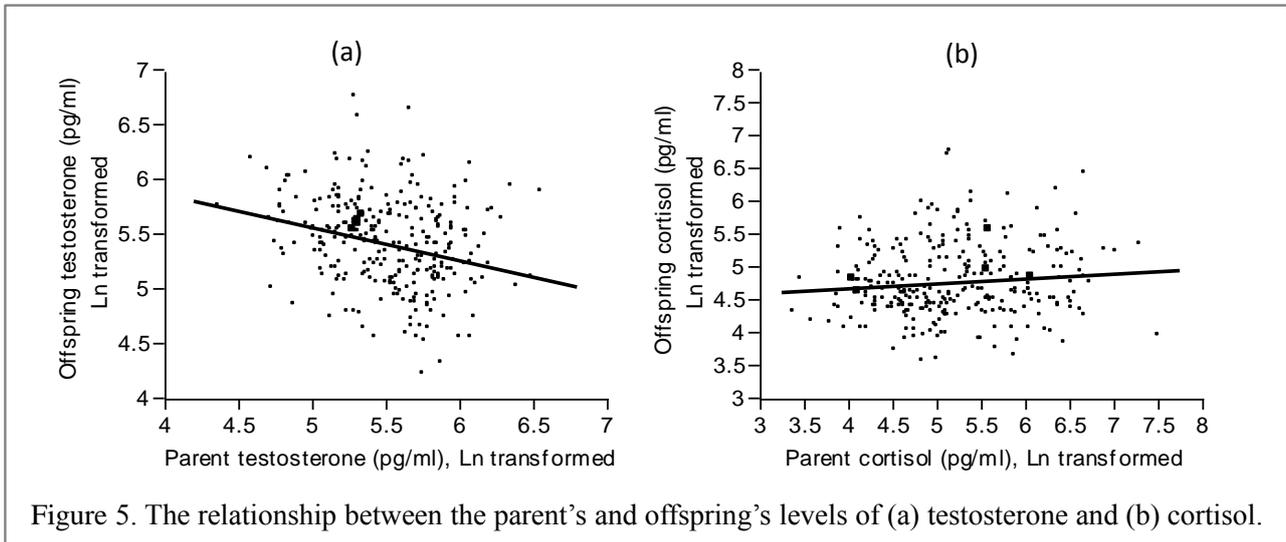


Figure 5. The relationship between the parent’s and offspring’s levels of (a) testosterone and (b) cortisol.

testosterone was, unexpectedly, negatively associated with those of the offspring ($b = -0.30 \pm 0.06$, $F_{1,286} = 22.5$, $p < 0.001$; Figure 6a). The parent’s levels of cortisol had a non-significant positive relationship with those of the offspring ($b = 0.07 \pm 0.04$, $F_{1,286} = 2.7$, $p = 0.097$; Figure 6b).

Importance of parent’s hormonal state to offspring’s aggressiveness:

Parent’s levels of testosterone ($b = -0.13 \pm 0.15$, $F_{1,286} = 0.7$, $p = 0.391$) and cortisol ($b = -0.08 \pm 0.07$, $F_{1,286} = 1.2$, $p = 0.265$) did not have significant relationship with offspring’s aggressiveness (i.e., pre-experience attack rate).

Importance of lineage to aggressiveness:

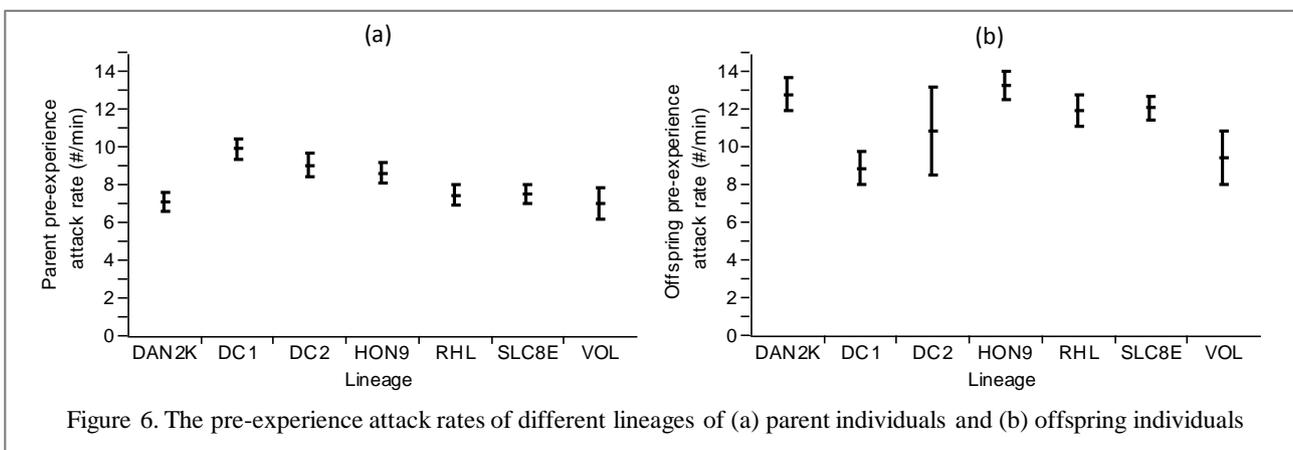


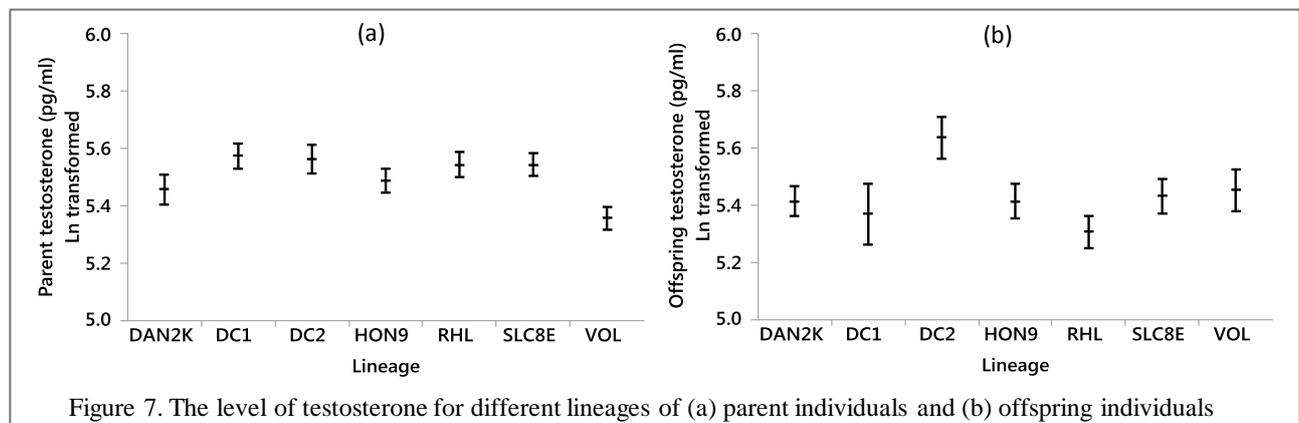
Figure 6. The pre-experience attack rates of different lineages of (a) parent individuals and (b) offspring individuals

Lineage had significant influence on both the parent’s ($F_{6,497} = 3.5$, $p = 0.002$) and the offspring’s ($F_{6,281} = 2.2$, $p = 0.0472$) aggressiveness (i.e., pre-experience attack rate). For the parent

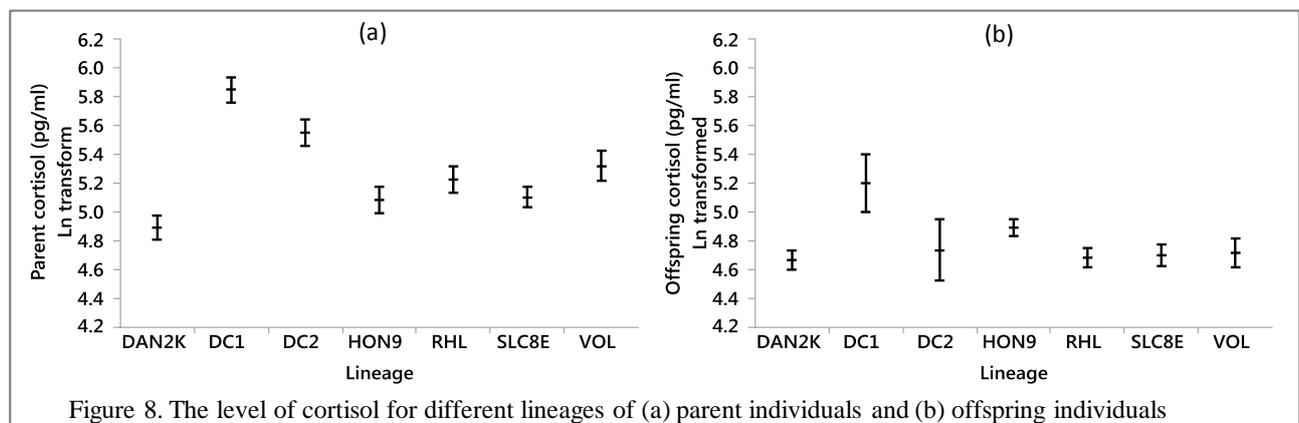
individuals, DC1 was the most aggressive lineage while VOL was the least aggressive lineage (Figure 6a). For the offspring individuals, however, HON9 was the most aggressive lineage while the DC1 became the least aggressive lineage (Figure 6b). This inconsistency in the aggressiveness among different lineage between the parents and the offspring was unexpected.

Importance of lineage to hormonal state:

Lineage had significant influence on the parent's ($F_{6,497} = 3.0, p = 0.007$) levels of testosterone. For the parent individuals, DC1, DC2, RHL and SLC8E had (significantly) higher testosterone levels than VOL ($p < 0.05$, Tukey multiple comparisons), while DAN2K and HON9 had intermediate levels (Figure 7a). For the offspring individuals, there was not overall difference in testosterone levels ($F_{6,281} = 1.0, p = 0.433$) (Figure 7b). Therefore, lineage did not seem to have consistent between-generation influences on the fish's testosterone levels.



Lineage had significant influence on both the parent's ($F_{6,497} = 12.9, p < 0.001$) (Figure 8a) and the offspring's ($F_{6,281} = 3.0, p = 0.008$) (Figure 8b) levels of cortisol. And, for both the parents and the offspring, DC1 had the highest while the DAN2K had the lowest cortisol levels (Figures 8a,b). Lineage thus had some consistent between-generation influence on the fish's cortisol levels.



Consistent with the above analyses, if we included lineage as a factor in general linear models, we found that parents' testosterone levels ($p < 0.001$), but not lineage ($p = 0.330$), significantly

explained offspring's testosterone levels (Table 8). On the other hand, lineage ($p = 0.018$), but not parents cortisol levels ($p = 0.410$) significantly explained offspring's cortisol levels (Table 9).

Table 8. The importance of parental levels of testosterone and lineage on offspring's levels of testosterone.

	<i>F</i>	<i>dfs</i>	<i>p</i>
Parental level	23.1	1,280	<0.001
Lineage	1.2	6,280	0.330

Table 9. The importance of parental levels of cortisol and lineage on offspring's levels of cortisol.

	<i>F</i>	<i>dfs</i>	<i>p</i>
Parental level	0.7	1,280	0.410
Lineage	2.6	6,280	0.018

Discussion

The fish exhibited between-individual differences and within-individual consistency in aggressiveness (in terms of attack rates), even for measurements that were taken more than 1-month apart. The fish thus exhibits “aggression personality” such that some of fish are consistently more aggressive and attack their own mirror images at higher rates than others. It is surprising that the fish exhibited higher consistency in behavioral traits (attack rate) over time than in physiological traits (levels of testosterone and cortisol), especially given that the fish’s aggressiveness was found to be positively related to the level of testosterone and cortisol in previous studies (Chang et al. 2012). These results suggest that the stability of the attack rate is likely mediated by physiological mechanisms other than the levels of testosterone or cortisol. Pre- and post-experience attack rates were positively correlated in both Studies 1 and 2, showing the robustness of the consistency in this fish’s aggressiveness. Although previous winning and losing experience could increase or decrease the fish’s aggressiveness, the effects were not enough to mask the consistency.

It was also surprising that neither winner nor loser effects were detected in Study 1, given that this fish has been shown to exhibit significant winner and loser effects in previous studies (Lan & Hsu 2011; Earley et al. 2013). The main difference between Study 1 and previous studies was that the fish faced its own mirror image in Study 1, but faced a conspecific rival in previous studies. Studies have shown that fish could potentially respond differently both behaviorally (Balzarini et al. 2014) and physiologically (Li et al. 2018) towards mirror images than towards true rivals. The validity of using behavioral responses toward mirror images as proxies for behavioral responses toward true conspecific rivals should therefore be evaluated (but not assumed) for each individual species. A recent study of our laboratory (Li et al. 2020) showed that the responses of *K. marmoratus* toward its own mirror images indeed predict its responses toward conspecific rivals. The attack rate in mirror test is therefore a reasonable index for the aggressiveness of *K. marmoratus*. And, we did detect significant loser effects in Study 2. For Study 2, to reinforce the influence of winning or losing experiences, the test fish were given the same (winning or losing) experience trainings twice (once a day for two consecutive days) instead of just once as in Study 1. In another study, we gave the fish the same experience three times (once a day for three consecutive days) and detected significant winner and loser effects using mirror tests. Overall, mirror tests provide reasonable tools for measuring aggressiveness and winner/loser effects in the fish. However, to more reliably detect winner and/or loser effects, multiple experience training sections might be necessary.

Although no winner or loser effects were detected in Study 1, the change in the attack rate after the first winning experience was related positively with the individual’s levels of testosterone and cortisol. It is intriguing that individuals with higher levels of testosterone or cortisol had a higher increase in attack rate after receiving a winning experience. Although these trends were not detected in the second experience trial (of Study 1), the importance of hormonal state to the fish’s responsiveness to winning and/or losing experience should be more thoroughly investigated.

Individuals that attacked their mirror images at higher rates prior to experience trainings (pre-experience attack rate) had lower attack rates after the experience trainings (post-experience attack rate). These trends suggest some kinds of upper ceiling for attack rates in the fish such that the attack rates for fish with higher pre-experience attack rates just could not go much higher.

We did not find consistent change in attack rates after a winning or losing experience in Study 1; the fish did not respond consistently to a winning or losing experience. These results should be interpreted with cautions nonetheless because we did not detect significant winner or loser effects in the Study. Given that the fish showed winner/loser effect toward its mirror image after receiving the same experience multiple (2 or 3) times, whether the fish responds consistently to winning or losing experiences should be examined again with revised experimental protocols by giving the fish the same experience 3 times.

In Study 2, the parent fish exhibited significant loser effects and attacked their mirror images at lower rates after receiving two consecutive losing experiences. Parent fish that received losing experience treatment exhibited lower consistency between the pre- and post-experience aggressiveness suggesting that different parent fish responded to the losing experiences differently.

Contrary to our expectations, offspring of parent fish that received losing experiences behaved more aggressively than the offspring of the parent fish that received winning or no contest experiences. In fact, offspring of the parent fish that received no contest experiences behaved the least aggressive. Perhaps parents' contest experiences (winning or losing) cause the offspring to behave more aggressively because they 'inform' the offspring the presence of competitors. The physiological mechanisms of how parents' experiences affect offspring's behaviors are unclear. Previous studies of the fish showed that parents' contest experiences do not alter the parent fish's testosterone or cortisol levels (Chang et al. 2012; Earley et al. 2013). The present study showed that parents' contest experience affected the offspring's attack rates but not their levels of testosterone or cortisol. Together, these results present strong evidence that hormones (testosterone and cortisol) are not responsible for winner or loser effects in the fish, despite that these hormones have close link to the fish's contest behaviors (Earley & Hsu 2008; Li et al. 2020). A previous study of the fish showed androgen receptor to be involved in mediating winner/loser effects (Li et al. 2014). It is therefore possible that androgen receptor also mediates the effect of parental contest experiences on offspring's aggressiveness. This hypothesis, nonetheless, needs to be examined.

In addition to affect offspring's aggressiveness, parents' contest experience also had some influences on the offspring's responsiveness to contest experiences. The offspring of parents that received winning experiences (but not losing or no contest experiences) exhibited significant loser effects (more responsive to losing experiences). Also, offspring of parents that received losing experiences tended to lower their attack rate after experience treatments, irrespective of the type of experiences that they received. Overall, losing experiences had profound effects on the fish; parent fish that received losing experiences (1) lowered their post-experience attack rate, and their offspring (2) attacked their mirror images at higher rates prior to experience treatments but (3) had greater decreases in their attack rates after experience treatments. Whether, and if so how, the

offspring responses to their parents' losing experiences help them to better adapt to their environment requires further investigation.

Parents' aggressiveness, responsiveness to contest experiences and cortisol levels did not predict their offspring's corresponding traits. Parents' testosterone levels, however, had a strong negative association with offspring's testosterone levels. Furthermore, although lineage affected parents' and offspring's aggressiveness (attack rates) and testosterone and cortisol levels, lineage did not have consistent influences on the parents and the offspring. These results suggest that these traits are highly affected by environmental factors and have low heritability.

Summary/Conclusions:

The results of this research project showed that (1) the attack rate and the levels of testosterone and cortisol are highly consistent in the fish, (2) the consistency in the attack rate was higher than the consistency in the hormone levels, (3) individuals of the fish did not appear to respond consistently to winning or losing experiences, (4) parents' contest experiences significantly affected offspring's aggressiveness and responses to contest experiences, (5) parent fish that received losing experiences had lower consistency in their aggressiveness, (6) parent's and offspring's levels of aggressiveness and hormones were not positive correlated, (7) lineage did not have consistent influences on parent's and offspring's levels of aggressiveness or hormones.

Overall, the fish's aggressiveness was consistent over time. This consistency was probably not mediated by the levels of testosterone or cortisol because the consistency in aggressiveness was higher than the consistency in the testosterone or cortisol levels. The physiological mechanisms important to the consistency in aggressiveness; therefore, remain unclear. We did not find the fish to respond consistently to a winning or a losing experience. This result should be interpreted with cautions, however, because no significant winner or loser effect was detected in Study 1. We recommend in the future, for each of the experience training trials, multiple experiences should be given to the test fish to reinforce the effects of the experiences. Although parents' contest experiences had important influences on the offspring's behaviors, the results that parents' behavioral and hormonal traits did not explain the corresponding traits in the offspring and that lineage did not have similar effects on the parents' and the offspring's traits suggest that the heritability of these traits are likely low.

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106年度專題研究計畫成果彙整表

計畫主持人：許鈺鸚		計畫編號：106-2621-B-003-001-MY3	
計畫名稱：打鬥勝敗經驗效應展現之差異性、一致性與母體效應			
成果項目		量化	單位 質化 (說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等)
國內 學術性論文	期刊論文	0	
	研討會論文	6	<p>第一年兩篇： 2018臺大師大生態演化學術聯合發表會暨壁報展(2018/05/27): (1) 潘俊穎、孫允哲、許鈺鸚：親代的勝敗經驗是否影響子代對於經驗的行為改變：以紅樹林鱗魚為例。(壁報) (2) 陳家平、許鈺鸚：動物年紀及荷爾蒙濃度對勝敗者效應之影響。(壁報)</p> <p>第二年三篇： 2019動物行為暨生態研討會 2019/1/21-22 國立臺灣大學，臺北，臺灣： (1) 孫允哲、潘俊穎、許鈺鸚：勝敗經驗對個體打鬥行為的改變是否具有一致性。(壁報) (2) 陳家平、許鈺鸚：內在因素及打鬥經驗如何影響個體攻擊性。(壁報) (3) 張毓庭、陳昱儒、曾庸哲、許鈺鸚：親代勝敗經驗對子代的行為、生理與生活史特徵的影響。(壁報- 獲得：優等獎)</p> <p>第三年一篇： 2020 動物行為暨生態研討會 2020/1/14-15 國立澎湖科技大學，澎湖，臺灣 (1) 潘俊穎、孫允哲、陳濤、許鈺鸚：親代的打鬥勝敗經驗是否會影響子代獲得打鬥勝敗經驗後的行為表現-以紅樹林鱗魚為例。(壁報)</p>
	專書	0	本
	專書論文	0	章
	技術報告	0	篇
	其他	3	篇

					<p>第三年：</p> <p>碩士論文兩份：</p> <p>1. 張毓庭 108年8月親代勝敗經驗對子代的行為、生理與生活史特徵的影響 (Yu-Ting Chang 2019 The importance of parent' s winning/losing experiences on individual' s behavioural, physiological and life-history traits)</p> <p>2. 孫允哲109年2月打鬥勝敗經驗效應展現之一致性(Yun-Jhe Sun 2020 Whether individuals respond consistently to winning and losing experiences)</p>
國外	學術性論文	期刊論文	0		
		研討會論文	3	篇	<p>第二年三篇：</p> <p>2018英國生態學會年會2018/12/16-19伯明罕國際會議中心，伯明罕，英國：</p> <p>1. Chen CP, Hsu Y. The importance of fighting ability, growth rate and age to the influence of winning/losing experience on aggressiveness. (poster)</p> <p>2. Chang YT, Chen YJ, Tseng YC, Hsu Y. The importance of parent' s winning/losing experiences on offspring' s behavioral, physiological and life-history traits. (poster)</p> <p>2019美國動物行為學會與國際行為學會連活國際會議(Meeting of the 56th Annual Conference of the Animal Behavior Society and the 36th International Ethological Conference. University of Illinois at Chicago, Chicago, USA)</p> <p>2019/07/23-27 伊利諾大學芝加哥分校，芝加哥，美國：</p> <p>3. Sun YJ, Hsu Y. (2019) Whether individuals respond consistently to winning and losing experiences.</p>
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
參與計	本國籍	大專生	2	人次	<p>第一年：</p> <p>大專生：2 (顏全佑、蕭羽庭)</p> <p>進行實驗、擷取資料、照顧實驗魚等</p>

畫 人 力		碩士生	18	<p>第一年：5人次（張毓庭、孫允哲、陳震邑、潘俊穎、林聖翔）</p> <p>第二年：7人次（張毓庭、孫允哲、陳震邑、潘俊穎、王筱萍、林柏毅、陳家平）</p> <p>第三年：6人次（孫允哲、陳震邑、潘俊穎、王筱萍、陳仙霈、沈至瑜）</p> <p>進行實驗、擷取/整理/輸入/分析資料、發表成果、照顧與繁殖實驗魚等</p>
		博士生	3	<p>第一年：1人次（陳昱儒）</p> <p>第二年：1人次（陳昱儒）</p> <p>第三年：1人次（陳昱儒）</p> <p>進行實驗、擷取/整理/輸入/分析資料、管理魚房、照顧與繁殖實驗魚等</p>
		博士級研究人員	0	
		專任人員	0	
	非本國籍	大專生	0	
		碩士生	0	
		博士生	0	
		博士級研究人員	0	
		專任人員	0	
	<p>其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）</p>			