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An Army of One: Predaceous Diving Beetle Life History Stages Affect Interactions With Shared Mosquito Prey

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Abstract

Predators with complex life cycles often differ in their morphology, behavior, and trophic position across their ontogeny, and may thus have variable effects on shared prey. We used the predaceous diving beetle *Laccophilus fasciatus rufus* as our predator, whose larvae and adults often co-occur in freshwater lentic systems. As a shared prey we used early and late instar *Culex quinquefasciatus*, a common wetland mosquito. We found that single adult predators were more likely to consume late instar prey compared to juvenile predators, who ate early and late instar prey equally. A mixture of juvenile and adult predator stages led to higher consumption of prey when compared to either predator type alone. Adult dytiscids consumed three times as many dead prey compared to living ones, thus implying a role in scavenging for this life history stage. Our work highlights that predators with complex life history stages may affect shared prey in complicated and unpredictable ways.

Keywords: complex life cycle, Culicidae, Dytiscidae, pond

Temporary lentic aquatic systems are often dominated by animals that exhibit complex life cycles, including anurans and many insects, however the interactions of these adult and juvenile predators with other species are not well studied. A complex life cycle is a maturation cycle in which different morphological changes occur during the growth of an organism (Wilbur 1980). Examples of animals that have a complex life cycle amphibians (e.g., frogs), many marine invertebrates, and many aquatic insects (e.g., Odonata, Coleoptera). Some insects exhibit indirect metamorphosis, wherein adults and juveniles are morphologically distinct, as opposed to direct metamorphosis where the emerging young are just smaller versions of the adults. Besides morphological changes, some animals with complex life cycles have larval and adult stages that occur in different habitats, have different diets, and are exposed to different interactions (predation, mutualisms) (Wilbur 1980). Predators with complex life cycles may exert different effects on a food web depending on their stage, and their effects may move beyond their current niche (McCoy et al. 2009).

Although common in lentic habitats, predaceous diving beetles (Coleoptera: Dytiscidae), have received little attention as predators. What is known is that they are important predators on both invertebrate and vertebrate prey (Culler et al. 2014). This highly diverse family of predators (~ 4,200 species worldwide) play a structurally important role in aquatic food webs (Thakare & Zade 2011) and are potentially important predators of many taxa, including mosquito larvae, in natural habitats (Larson et al. 2000), especially in fishless systems (Batzner & Wissinger 1996). Adult dytiscids are often capable of flight and move among isolated bodies of freshwater, using vision, chemoreception, and vibration to recognize and choose their prey (Hagen et al. 1976). When comparing them to their juvenile counterparts, whom are obligate aquatic predators requiring prey movement to elicit a response, adult dytiscids are not adept hunters (Johnson et al.

2003). Unlike adults, juveniles have also been seen to exhibit a variety of different predatory behaviors including sit-and-pursue, sit-and-wait, and active hunting modes (Young 1967, Formanowicz 1982, Yee 2010). Regarded as “clumsy”, the adults may select prey that is easy to capture, including dead or dying individuals; scavenging on dead material that may be energetically favorable (Bosi 2001). Adults and juvenile beetles often co-occur in the same habitats (Larson et al. 2000), however it is unknown how the different stages within each species differ with respect to predation.

Mosquito larvae are thought to be an important food item for many aquatic animals, including many species of predaceous diving beetles (summarized in Culler et al. 2014, Shalan & Canyon 2009). Since the 1990s, insecticides have become a widely acceptable method of mosquito control, but resistance to these chemicals have been a challenge to effective control measures (Brogdon & McAllister 1998). As a consequence of this resistance, aquatic ecologists see the necessity to study the use of natural predators as a biocontrol on mosquito populations. Research on larval mosquito population control via aquatic predators has become of recent interest, however the focus on these investigations has been on factors like prey stage (Chandra et al. 2008), habitat characteristics (Ohba & Ushio 2015), and prey type (Culler & Lamp 2009). Currently, no studies have investigated the effects of different predator life-history stages on consumption of mosquito prey.

We conducted a series of experiments to better understand the contribution of both the adults and juveniles of the beetle *Laccophilus fasciatus rufus* on prey populations of the mosquito *Culex quinquefasciatus*. *Laccophilus fasciatus rufus* is a common dytiscid in North America (Larson et al. 2000) and has been shown to prey on mosquitoes (Pitcher & Yee 2014). *Culex quinquefasciatus* (southern house mosquito) is a common open water mosquito that has a world-

wide distribution, and has been used as a prey species of dytiscid predation elsewhere (Aditya et al. 2006, Chandra et al. 2008). Specifically, our objectives in this study were to 1) quantify consumption rates of *L. f. rufus* adults and juveniles on different stages of mosquito larvae under different prey and plant densities, 2) examine if combinations of predator life-history stages affected predation rates compared to single predator stages, and 3) test adult dytiscid prey preference between living and dead prey. We hypothesized that because of potential differences in hunting behavior and other life-history differences (Yee 2010), adult and juvenile predators will display different effects on prey populations. For instance, as adults are more likely to exhibit searching behavior (Larson et al. 2010) and some juveniles are known to be sit-and-wait hunters (Yee 2010), we may predict that adults would consume more prey given the potential for higher encounter rates, and that these differences may also influence predation rates in single versus multi-predator trials. Studying such interactions will improve the understanding of the effect of the complex life history of predators on shared prey in general, and will lead specifically to a better understanding of the bio-control abilities of dytiscids in natural environments.

Materials and Methods

***Laccophilus fasciatus rufus*.** No taxonomic keys exist to distinguish different species of *Laccophilus* larvae (hereafter, juveniles, to distinguish from mosquito prey). Thus, to assure that the correct species of *Laccophilus* was used, breeding of adults in a controlled setting was necessary. Specifically, adult *L. f. rufus* were collected from aquatic habitats in and around Hattiesburg, MS (31°19'38" N, 89°17'25" W). Males and females were placed in large plastic tubs (91.5 x 61 x 20.3 cm) containing aquatic plants, pond water, food, and substrate. Plants

(*Ludwigia palustris*) were collected from ponds locally and rinsed repeatedly to remove invertebrates. Tubs were covered with no-see-um mesh and left for several weeks at the USM Science Park, located approximately 8 km east of the Hattiesburg campus, during which time any juveniles that were produced were removed for experiments (genus level identifications were made using Larson et al. 2000). For experiments requiring adults, additional *L. f. rufus* were collected from aquatic habitats and isolated in separate plastic cups containing 100 mL filtered pond water and a wooden perch. Adults and juveniles were fed once daily using frozen chironomid larvae or live mosquitoes until experimental trials began. All juveniles used in the experiments were either 2nd or 3rd instars. Predation by either adults or juvenile predator stages were easily distinguishable based on known behavior: mosquitoes killed by adults were wholly consumed or dismembered, whereas juveniles pierce and suck prey leaving only an empty exoskeleton. Prey (*Culex quinquefasciatus*) were obtained from laboratory colonies (F₄ or less) that were originally collected as egg rafts from areas near campus.

Experiment 1. Predator stage and consumption of different prey stages. To quantify predation rates on shared mosquito prey, both juvenile and adult beetles were used separately in feeding trials. Feeding trials were conducted in small plastic aquaria (20.3 x 15.2 x 12.7 cm) filled with 3.9 L filtered pond water collected from ponds in the study area. In each aquarium, three different levels of plant stem density were used: 0, 3, and 6 stems (hereafter no, low, and high plant densities). We used *Ludwigia palustris*, a plant that is commonly found in dytiscid habitats in the study area at variable densities (Pitcher & Yee 2014). Testing predation on mosquitoes under different plant densities has been shown to affect predator-prey interactions (Savino & Stein 2011, Yee, 2010) and we predicted that increasing plant density would increase refugia for prey and thus reduce predation rates. Prey density trials consisted of three levels: 5,

10, or 15 of either early (1st and 2nd) or late (3rd and 4th) instar *Culex quinquefasciatus*, hereafter low, medium, and high prey. In some lentic systems different levels of mosquito prey can also affect predation rates (Alto et al. 2012). *Culex quinquefasciatus* is often found in open water (Vinogradova 2000) and often cohabitates with these predators (unpublished data). Inclusion of different sized prey let us examine how predator life stages affect predation across prey life history stages. Plant density (3), prey density (3), and prey stage (2) were crossed to yield 18 combinations that were replicated 5 times for each predator stage. Uneaten mosquitoes were counted after 4 hrs in each aquarium. Aquariums were placed in an incubator at 27° C on a 14:10 (dark:light) photoperiod (approximate summer conditions for the study area).

To assess differences in prey consumption a four-way analysis of variance (ANOVA) was used to determine if differences existed among the different plant densities (3), prey densities (3), prey stages (2), and predator stage (2). A $\log_{(x+1)}$ transformation was applied to the raw data to meet assumptions of normality. Tukey's test were then conducted to assess differences among specific treatment levels while controlling for experimental-wise error rates. All statistical analyses were conducted in SAS (SAS 2004).

Experiment 2. Single and multiple predator stages and prey consumption. We examined how single and mixed predator life history stages affected consumption of early and late instar prey. Specifically, we placed either two adult predators, two juveniles, or one of each into aquaria established in the same manner as described above, with 10 prey of each stage offered in the single prey treatment levels and 10 of each in the combined level. For these trials, beetles were starved 24 hrs prior to introducing them into aquaria to standardize hunger levels; trials ran for four hours. We discarded one replicate of the mixed predator treatment level from analysis as the adult beetle killed the juvenile. We also prepared 6 replicates of two juveniles and a juvenile

and adult predator without prey to evaluate potential predation among predators (we assumed that adults were unlikely to kill one another) and three replicates with no predators (control) and collected data as above.

A two-way ANOVA was used to assess differences in consumption of different prey stages (early, late) across predator combinations (juvenile alone, adult alone, juvenile and adult). Raw data met assumptions and differences among levels of significant treatment effects were assessed using a Tukey adjustment.

Experiment 3. Adult predator selection of prey. Experiment 1 did not produce differences in predation rates among prey densities or plant densities (see Results). These treatments were subsequently excluded from this study, and we used three sprigs of plant for all replicates. Furthermore, only late instar prey were utilized as adult dytiscids were observed to consume significantly more late compared to early instar mosquito larvae. Specifically, we used three different prey treatment levels to examine prey preference for adult beetles only: 10 dead mosquito larvae, 10 living larvae, and 5 dead and 5 living. Larvae were freshly killed by placing them in water in a microwave for approximately 1 minute. Each treatment level was replicated six times yielding 24 experimental units. All other aspects of this experiment (e.g., aquarium size, time until data collected) were the same as the ones described above.

A one-way ANOVA was used to test for differences between different prey treatment levels (percent of prey eaten by adult dytiscids in the living, dead, of the combination of living and dead treatment levels). Raw data met assumptions of the analysis.

Results

Experiment 1. Predator stage and consumption of different prey stages. We detected significant effects of predator stage and a prey stage by predator stage interaction; other effects

were not significant (Table 1). Specifically, adult beetles consumed more late instar mosquitoes compared to juvenile beetles, whereas both predator stages consumed a similar but lower number of early instar mosquitoes (Fig. 1).

Experiment 2. Single and multiple predators stages and prey consumption. No prey died in the no predator controls, however in two replicates of the controls without prey juveniles were killed by adults (33%). About 30% more prey were consumed when an adult and juvenile were together than when either stage was alone (Fig. 2A) ($F_{2,46} = 6.01$, $P = 0.005$). The highest consumption was on early instar prey offered alone, followed by either early instars in combination with late instars or late instars by themselves (Fig. 2B) ($F_{3,46} = 31.51$, $P < 0.001$). Late instar prey offered with the early stage were consumed least overall (Fig. 2B). The interaction between predator combination and prey combination was not significant ($F_{6,46} = 1.99$, $P = 0.087$).

Experiment 3. Adult predator selection of prey. Adults ate three times as many dead prey compared to living prey when both types were offered simultaneously (Fig. 3) ($F_{3,16} = 5.944$, $P = 0.007$). In addition, the overall consumption of live or dead prey did not vary when these types were offered alone or in combination, indicating that prey density did not affect prey selection.

Discussion

It has been assumed that predators species are interchangeable in their effects on prey (Fretwell 1987), however such an assumption is likely wrong, given that different predators may act in dissimilar ways to enhance or weaker overall predation pressure in food webs (Schmitz 2007). However, little attention has been paid to predators with complex life cycles, wherein different life history stages may exert different forces on shared prey. Our goal was to test for

consumption differences for adults and juveniles of *L. f. rufus* on a shared prey, which would allow us to examine the specific nature of predation effects of a predator with a complex life cycle on a shared prey. Our hypothesis that adult and juvenile predators would display different effects on prey populations was generally supported. We found that adult *L. f. rufus* consumed more late instar mosquito larvae compared to juveniles; adults also consumed more dead than living prey. The higher consumption of larger prey by adult predators may be due to energetic differences, as larger prey are likely an easier target for adults compared to juvenile predators and have a higher caloric value. Adult beetles are fast-moving adept swimmers, whereas larvae generally walk along the bottom or on surfaces and often sit and wait for prey, and thus capture of larger prey is likely easier for adults compared to juveniles. However, we found no differences in predation on early instars, suggesting that both juveniles and adults can equally affect small mosquitoes. Although we found no significant differences between prey sizes within each predator stage consumption of early and late instars for adult predators did approach significance ($P = 0.084$), whereas juveniles did not ($P = 0.180$). This would seem to bolster the possibility that the larger, more active adult predators are more adept in consuming larger versus smaller prey. At present, no other study has compared predator consumption for different dytiscid life history stages across prey sizes within a prey species. Aditya and Saha (2006) did find that feeding rates of adult *Rhantus sikkimensis* were higher on small chironomid prey versus larger ones, a finding that is opposite of ours. Moreover, Lundkvist et al. (2003) found differences in mosquito size preferences for three species of dytiscids, and concluded that there should exist an inverse relationship between predator size and prey size, a prediction our data does not support.

The differences between consumption by beetles for a single prey type in our study are broadly consistent with work by Klecka and Boukal (2012) who showed that adult and juvenile

dytiscids (*Acilius* sp.) exhibited preferences for different prey, with juveniles preferring *Culex* sp. and adult beetles preferring chironomids. This could be explained by differences in prey movement and location. Because of a lack of refugia, chironomids were an easy target for predators, and likely required low effort by adults to obtain them (Klecka and Boukal 2012). Regardless of the mechanism, such ontogenetic diet shifts (ODS) might be expected as a way to reduce intraspecific competition, and would also be predicted when animals change in morphology, size, and behavior over their life cycle (Ohba 2009, Klecka & Boukal 2012). Such shifts may not always be perfect, allowing a degree of overlap that would bring adults and juveniles into direct competition. Indeed, Klecka and Boukal (2012) did show that diet overlap was pronounced for life history stages in two species of dytiscids, but did not offer any mechanism for how species may limit intraspecific competition. Instead of dampening competition, we suggest that with greater diet overlap there may be more opportunities for predation among the predators (i.e., intraguild predation (IGP)). We found some evidence for IGP, but not symmetrical, as juveniles succumbed to predation by adults one third of the time in the absence of prey and in one of our replicates when prey were present. As IGP (e.g., Nilsson & Söderström 1988) and instances of cannibalism (e.g., Dending 1988, Hicks 1994, Yee, 2010) exist among dytiscids, these interactions could further complicate the way in which changes in predator life history may interact with prey, to perhaps change overall predation rates among life history stages.

We did determine that the combined feeding of an adult and juvenile predator increased prey consumption 30% over single predator stage trials. One explanation for this increased feeding is due to predator spatial niches and foraging behavior. Adult *L. f. rufus* are active predators (Pitcher & Yee 2014) whereas *L. f. rufus* larvae are mostly sit-and-wait predators (personal

observation); adults also move actively through the water column, whereas juveniles of this species remain relatively motionless at the bottom or on plants. This suggests inherent spatial differences among life history stages, and coupled with differences in how these predator stages feed, may help to explain the synergistic effects with mixed versus single predator stages for dytiscids. Such behavioral differences among dytiscid larvae in feeding mode and hunting domain have been documented for larval dytiscids (Yee 2010), however this is the first study to look at the potential role of larvae and adults to affect shared prey. One consequence of this work is to suggest that there may be compounding effects upon prey populations in nature by predators with complex life cycles (Wilbur 1980). To determine if this is the case, additional experiments, wherein adult and juvenile predators of various densities are used, will need to be conducted.

Unintentionally, we also tested the effect of multiple predators of each prey stage. In Experiment 1, single adult beetles ate more large prey but there were no differences between prey sizes within beetle stage (Fig. 1), whereas in Experiment 2 when two adult predators were tested they generally ate more early instars (predator effect combines data from adults and juveniles, Fig. 2B). The experimental design was not identical between trials, however differences in plant density and prey density that were investigated in Experiment 1 were non-significant, and thus were eliminated in Experiment 2. Given this, differences in consumption would seem to point to potential interactions between predators, an effect found in some other studies. Aditya and Saha (2006), who tested predation of adults of the dytiscid *Rhantus sikkimensis* at two different predator densities on mosquito and chironomid prey, noted significant differences in prey consumed with two predators consuming more prey than three. However, Chandra et al. (2008) found higher predation rates of two juvenile *Acilius sulcatus* feeding on *C. quinquefasciatus* compared to one juvenile, although variation in prey

consumption was also affected by prey density and volume. The interaction of multiple predators on shared prey is understudied in aquatic insects in general, and dytiscids specifically, but as the presence of multiple predators is the norm in natural temporary water bodies such data that explores predator density are needed.

We also showed that adult *L. f. rufus* ate more dead mosquitoes compared to live ones. If adult dytiscids are a good bio-control agent then we would expect living prey to be preferred when given the choice between dead and living mosquitoes. Velasco and Millian (2008) found that adult dytiscids chose to scavenge on dead animal material rather than to hunt living prey. A similar result was observed by Kehl and Dettner (2003) who also tested prey preference by giving the dytiscid *Scarodytes halensis* mixed treatments of dead and living plankton (Copepoda and Cladocera). In our experiment, adult *L. f. rufus* had the ability to attack living prey when given no choice, but attacked fewer living prey when dead prey were available. Scavenging on dead animal material is common in dytiscids (Hicks 1994, Larson et al. 2000), and could be favored if it requires lower energy expenditure. Scavengers (especially ones that are capable of flight) can potentially transfer energy between adjacent ecosystems (Payne & Moore 2006). Considering the prevalence of this species in local water bodies (Pitcher & Yee 2014), the rate at which it scavenges, and potential mobility, this could possibly impact nutrient cycles in areas that they inhabit, although such speculation requires more testing.

Our results showed that beetles of both stages caused a significant decrease in mosquito densities, and support the results of others for dytiscids' ability to decrease mosquito larvae populations (e.g., Formanowicz 1982, Culler & Lamp 2009, Chandra et al., 2008). For example, Chandra et al. (2008) observed that in a 24 hr period, juvenile *Acilius sulcatus* (Coleoptera: Dytiscidae) ate on average 34 out of 200 late instar *C. quinquefasciatus* (~17%). The juvenile

dytiscids used in our experiment consumed 28% of the late instar *C. quinquefasciatus* larvae offered, even though the amount of time for this study's trials were shorter than those by Chandra et al. (2008). It is still unclear the degree to which adult dytiscids or larvae are important predators on mosquitoes in natural wetlands (Lundkvist et al. 2003, Shaalan and Canyon 2009), however it is likely that they do represent an important predator under a variety of circumstances, especially in fishless environments (Larson et al. 2000). Many different control agents are utilized for population suppression of mosquito larvae including vertebrate predators such as non-game fish (Kern 2004). In an experiment that tested prey preference of dytiscids and mosquito fish it was observed that mosquito fish did not show a preference for mosquitoes, however dytiscids did (Culler & Lamp 2009). Compilation of knowledge surrounding different species of dytiscids may allow researchers to construct better bio-control strategies that are more efficient than single species approaches.

Plant density had no significant effect on consumption of mosquito prey. Different plant densities were utilized because we predicted that higher plant densities would provide refugia for prey. Elsewhere (Yee 2010), the presence of plants altered the behavior of larval dytiscids when attacking their prey. One possible explanation for the lack of an effect of plants could be that the plant stem densities used may not have been appropriate to allow prey to use as shelter or as a vantage point for predators. Another explanation could be that *Culex* sp., including *C. quinquefasciatus*, are open water dwellers and would not utilize plant refugia (Clements 1999). Thus, even when offered plants as shelter, they may aggregate in the open water areas of the habitat, where they would be vulnerable to predators. However, mosquitoes staying at the surface regardless of plant density may also be a form of anti-predator behavior (Ohba & Ushio 2015). Such interactions between habitat factors, prey type, and predator life history and species in

dytiscids have not been explored in detail.

We detected no significant relationship of prey density on consumption rates. Formanowicz (1982), also did not find prey density to affect consumption rates across five different prey densities for three different species of juvenile dytiscids. He also noted no difference between predator searching times, handling time, and prey densities. In this case, it did not seem to matter what density of prey that was administered, suggesting that the dytiscids take the same amount of time to recognize, handle, and consume each prey. Additionally, Formanowicz (1982) attributed his results to the many different ways that juvenile dytiscids can hunt for prey. For instance, at low densities of prey juveniles would actively search at all times, whereas in high densities of prey they utilized a sit and ambush tactic (Formanowicz 1982). Others have found that prey density affects consumption of prey (Aditya & Saha 2006), however explanations for why this might occur are lacking.

It is clear that adult and juvenile *L. f. rufus* have different effects on a shared mosquito prey. The compounding effect that both life stages of dytiscids may have on shared prey populations is an underexplored area of research, but could be useful in understanding the degree to which dytiscids serve, for instance, as biocontrols of mosquitoes in natural wetlands. Moreover, research on intraguild predation would also be a necessary area of inquiry, where we hypothesize that adult dytiscids would play the role top predator, the juvenile as the intermediate prey, and the mosquito larvae as the basal resource. Our work points to an asymmetry in predation for different life-history stages of *L. f. rufus*, an area that has not been identified previously.

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462 **Table 1.** Results of 4-way ANOVA showing effects of predator stage (juvenile, adult), prey
 463 stage (early or late instar mosquito larvae), plant density (low, medium, high), and prey density
 464 (5, 10, or 15 mosquito larvae) and all their interactions on consumption rates of mosquito prey.
 465 Significant effects are presented in bold.

Factor	df	F	p-value
Predator Stage (D)	1, 108	4.48	0.0367
Prey Stage (P)	1, 108	0.02	0.8835
Plant Density (L)	2, 108	0.13	0.8823
Prey Density (S)	2, 108	0.11	0.8919
D x P	1, 108	9.48	0.0026
D x L	2, 108	0.13	0.8782
D x S	2, 108	0.09	0.9163
P x L	2, 108	0.24	0.7892
P x S	2, 108	1.67	0.1926
L x S	4, 108	0.39	0.8122
D x P x L	2, 108	0.37	0.6898
D x P x S	2, 108	0.08	0.9222
D x L x S	4, 108	0.28	0.8895
P x L x S	4, 108	0.29	0.8839
D x P x L x S	6, 108	0.08	0.9901

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Figure legends

Fig. 1 Results of the significant interaction between predator stages (juvenile and adult beetles) and prey stages (early and late instar *Culex quinquefasciatus*) on mean ($\pm$ SE) consumption rates. Means that do not share a letter are significantly different based on mean separation with a Tukey correction.

Fig. 2 Effects of single and combined predator life history stages on different stages of shared prey, *Culex quinquefasciatus*. A) Significant effect of predator stage (2 juvenile, 2 adult, or 1 of each *Laccophilus fasciatus rufus*) on mean ($\pm$ SE) percent consumption of larval *Culex quinquefasciatus*. B) Significant prey stage effect for early (1st, 2nd) and late (3rd, 4th) instars of prey offered either alone or in combination. In all panels means that do not share a letter are significantly different based on mean separation with a Tukey correction.

Fig. 3 Results of a one-way ANOVA on mean ($\pm$ SE) consumption of mosquitoes by adult dytiscids. Treatment levels included either 10 dead, 10 live, (noted as “10 prey” in the legend) or a mixture of 5 living and 5 dead late instar mosquitoes (*Culex quinquefasciatus*). Means that do not share a letter are significantly different based on mean separation with a Tukey correction.

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508 Fig. 1

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Fig. 2

Fig. 3