Prey abundance and intraguild predation between *Adalia bipunctata* (Coleoptera: Coccinellidae) and *Macrolophus pygmaeus* (Hemiptera: Miridae)

VINCENZO TROTTA, JULIANA DURÁN PRIETO, PAOLO FANTI and DONATELLA BATTAGLIA

Dipartimento di Scienze, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy;
e-mails: vincenzo.trotta@unibas.it; juliana.duran@unibas.it; paolo.fanti@unibas.it; donatella.battaglia@unibas.it

Abstract. *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) are two predatory insects commonly used as biological control agents. In order to determine the incidence with which both species attack and eat each other [Intraguild predation (IGP)], the direction and symmetry of the interaction between *A. bipunctata* and *M. pygmaeus* were characterized. In addition, whether the intensity of IGP between these two predators increased when the number of extraguild prey *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) decreased, was also determined. Unidirectional sensu stricto IGP between *A. bipunctata* and *M. pygmaeus* was recorded: when IGP occurred, *A. bipunctata* was always the IG predator that killed and ate *M. pygmaeus*, the IG prey. However, the intensity of IGP was a function of the abundance of the extraguild prey, *A. pisum*, since IGP increased when the number of extraguild prey decreased. These results are discussed in terms of theoretical models that predict stability and the outcome of using natural enemies to control pests.

INTRODUCTION

Intraguild predation (IGP) is a type of omnivory, which is defined as the "killing and eating species that use similar, often limiting, resources and that are potential competitors" (Polis et al., 1989; Polis & Holt, 1992). In IGP there are three organisms that interact: a natural enemy (the intraguild (IG) predator) that negatively affects a second natural enemy (the IG prey) by eating it and the shared (extraguild) prey (Rosenheim et al., 1995; Lucas, 2005).

IGP appears to be widespread in communities of biological control agents and may affect the distribution and abundance of IG competitors and modulate the dynamics of a pest population (Polis et al., 1989; Polis & Holt, 1992; Rosenheim et al., 1993, 1995; Lucas et al., 1998; Rosenheim, 1998; Colfer & Rosenheim, 2001). IGP is characterized by evaluating its intensity (the level of IGP in a specific combination of predators), its direction (identity of the predator and prey) and its symmetry (in asymmetrical IGP one species consistently preys upon the other, while in symmetrical IGP both species prey equally upon each other) (Lucas et al., 1998; Lucas, 2005).

If an IG predator encounters both IG prey and extraguild prey in the same patch, it is possible that the intensity of IGP depends on the abundance of extraguild prey. A model has been proposed in which the IG predator can switch from feeding exclusively on the most profitable prey (usually the extraguild prey) to feeding on the less profitable prey (the IG prey) when the encounter rate with the profitable prey falls below a certain threshold value (Křivan & Diehl, 2005). This model predicts that the coexistence of IG predators and IG prey is increased, even if the relative abundances of the organisms involved fluctuate over time (Law & Blackford, 1992; Křivan & Diehl, 2005). In biological control, if the IG predator is the superior competitor, theory predicts that the IG prey will always be excluded since the IG predator reduces the pest densities to lower densities than the IG prey, without IGP having a negative effect on biological control (Janssen et al., 2006). If this is correct then there is no advantage in using two species of natural enemies that are IGPs.

In the field, most aphid colonies are exploited simultaneously by several aphidophagous predators that eat each other, which in turn, influence the ecological and evolutionary traits of the different protagonists (Polis et al., 1989; Polis & Holt, 1992; Holt & Polis, 1997; Lucas, 2005; Yano, 2006). In this study we used *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) as model organisms in order to test whether or not the intensity of IGP (if any) is affected by the abundance of *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) (the extraguild prey). *A. pisum* is the main pest of forage crop such as pea, broad bean, clover and alfalfa. The heteropteran predator *M. pygmaeus* is a generalist zoo-phytophagous insect used broadly as a biological control agent in field and protected horticultural crops in the Mediterranean region (Castañé et al., 2004; Alomar et al., 2006). The two-spot ladybird beetle *A. bipunctata* is an aphidophagous predator, which occasionally feeds on other small insects (Hodek, 1973), and occurs in Europe, Central Asia, North America and Japan (Majerus, 1994; Sakuratani et al., 2000; De Clercq et al., 2005; Omkar & Pervez, 2005). The existence of IGP between *A. bipunctata* and other aphidophagous insects such as *Harmonia axyridis* (Pallas) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) is documented (Hemptinne et al., 2000; Burgio et al., 2005; Santi & Maini, 2006; Ware et al., 2008; Raak-van den Berg et al., 2012; Rondoni et al., 2012) as well as between *M. pygmaeus* and other entomophagous insects like syrphids, *Orius majusculus* (Hemiptera: Anthocoridae) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) (Jakobsen et al., 2004; Fréchette et al., 2007; Moreno-Ripoll et al., 2014; Perdikis et al., 2014). However, until now, IGP between *M. pygmaeus* and *A. bipunctata* has not been studied.

MATERIAL AND METHODS

Insect cultures

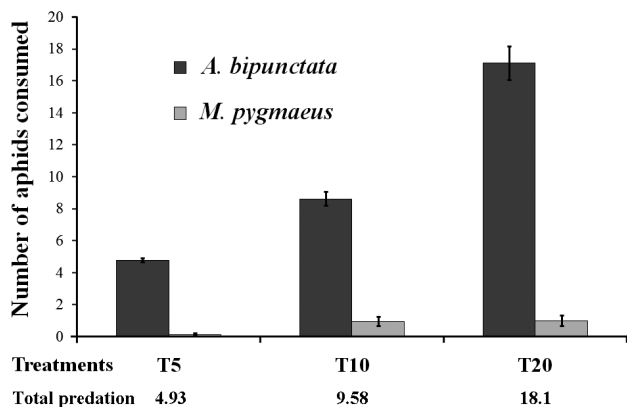*A. pisum* [collected near Salerno, Italy (40°37′01″N, 15°3′23″E)] were reared in an environmental chamber at 22°C,

Fig. 1. Number of aphids (mean ± standard errors) consumed by *A. bipunctata* (dark grey) and by *M. pygmaeus* (light grey) in each of the three treatments. Treatments: T5: one adult female of *M. pygmaeus* (*Mp*) plus one 4th instar larva of *A. bipunctata* (*Ab*) plus five nymphs of *A. pisum* (*Ap*) (N = 29); T10: one *Mp* plus one *Ab* plus ten *Ap* (N = 26); T20: one *Mp* plus one *Ab* plus twenty *Ap* (N = 20). Total predation is the total number of aphids consumed by both predators.
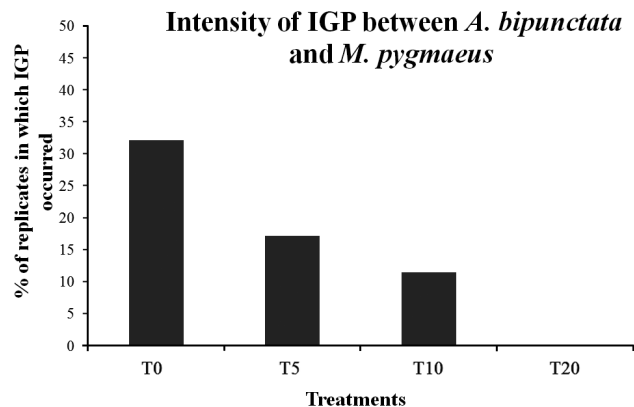
Fig. 2. Percentage of the replicates in each treatment in which IGP of *M. pygmaeus* by *A. bipunctata* was recorded. Treatments: T0, one adult female of *M. pygmaeus* (*Mp*) plus one 4th instar larva of *A. bipunctata* (*Ab*) and no nymphs of *A. pisum* (*Ap*) (N = 28); T5: one *Mp* plus one *Ab* plus five nymphs of *Ap* (N = 29); T10: one *Mp* plus one *Ab* plus ten *Ap* (N = 26); T20: one *Mp* plus one *Ab* plus twenty *Ap* (N = 20).

80–90% rh and a 18L: 6D photoperiod, feeding on broad bean plants (*Vicia faba* c.v. *Agua dulce*).

Macrolophus pygmaeus individuals came from a colony kept under laboratory conditions on potted tomato and broad bean plants, at room temperature and under a 18L: 6D photoperiod. Sterilized *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, were used as a supplemental diet. *Adalia bipunctata* was laboratory reared on *A. pisum*. The *E. kuehniella* eggs, *M. pygmaeus* and *A. bipunctata* were purchased from Koppert Biological Systems.

Experimental procedures

All the experiments were carried out in plastic cylinders (diameter: 5.5 cm, height: 7.5 cm, volume: 150 ml) with a mesh covered ventilation hole (4.5 cm in diameter) in the screw-top. Each cylinder contained a fresh cut broad bean leaf with the stalk inside an Eppendorf tube filled with water and sealed with parafilm to prevent desiccation. Cohorts of 2nd instar *A. pisum* were used in the experiments. Four different experimental treatments (T), always containing one *M. pygmaeus* adult female plus one *A. bipunctata* 4th instar larva, were set up as follows: T0: without *A. pisum* nymphs (N = 28 replicates); T5: with five *A. pisum* nymphs (N = 29); T10: with ten *A. pisum* nymphs (N = 26); T20: with twenty *A. pisum* nymphs (N = 20). For each experimental treatment, *M. pygmaeus*, *A. bipunctata* and *A. pisum* (if any) were gently transferred using a paintbrush into the cylinders with a broad bean leaf. The cylinders were kept in an environmental chamber at 22°C, 80–90% rh and a 18L: 6D photoperiod. After 24 h the cylinders were inspected in order to determine if IGP occurred; the number of aphids consumed by *A. bipunctata* and by *M. pygmaeus* was also recorded (in the latter case aphid skins remained after predator feeding).

Data analyses

The numbers of aphids consumed by *A. bipunctata* and *M. pygmaeus* at the three prey densities (5, 10 and 20 aphids) were separately analyzed using a one-way ANOVA model with treatment as the main factor. The number of replicates in which IGP occurred was analyzed using a Chi-square contingency table to determine if the intensity of IGP depended on the number of aphids. The analyses were done using R.3.1.1 software (R Core Team, 2014).

RESULTS

Extraguild prey consumption

We found that the number of aphids consumed by *A. bipunctata* significantly increased with the increase in the number of prey ($F_{2,72}$ = 117, $P < 0.001$; Fig. 1).

We also found a significant difference in the number of aphids consumed by *M. pygmaeus* in the presence of *A. bipunctata* at the three different aphid densities ($F_{2,72}$ = 4.5, $P < 0.05$), with the results for T10 and T20 treatments differing from T5 (Fig. 1).

The total number of aphids consumed by the two predators in the T5, T10 and T20 treatments was, respectively, 4.93, 9.58 and 18.1, which are very close to the total number of prey items in each of these treatments.

Intensity of the IGP of *M. pygmaeus* by *A. bipunctata* at different aphid densities

When IGP occurred, *M. pygmaeus* was always the IG prey and *A. bipunctata* the IG predator (asymmetrical IGP). The intensity of IGP was statistically different at the different aphid densities ($\chi^2_{(3)}$ = 9.4, $P = 0.02$), showing a clear negative trend from T0 (32%) to T20 (0%) (Fig. 2).

DISCUSSION AND CONCLUSIONS

The organisms that are involved in IGPs do not have well defined roles because several factors, such as their relative size, mobility, density, feeding specificity and morphology, influence whether they are predators or prey (Polis et al., 1989; Lucas et al., 1998; Burgio et al., 2005; Lucas, 2005; Fréchette et al., 2007). Based on the definition proposed by Lucas (2005), we demonstrated that there is unidirectional sensu stricto IGP between *A. bipunctata* and *M. pygmaeus*: when IGP occurs, *A. bipunctata* is always the IG predator that kills and eats *M. pygmaeus*, the IG prey. Similar to our results, Lucas & Alomar (2000, 2001) show that *M. pygmaeus* is frequently an IGP with *Dicyphus tamaninii* Wagner (Heteroptera: Miridae) as the IG prey.

The small volume of the arena used in the present experiment may have altered the behaviour and interaction between the species. It is known that the size of the arena has a clear effect on the intensity of IGP, with smaller and simpler arenas generating higher levels of IGP by increasing predator encounter rates and/

or by reducing refuge patches (Chang, 1996; Hindayana et al., 2001; Janssen et al., 2006; Ingels & De Clercq, 2011). However, the shape and dimensions of the arena allowed the mirid to escape predation by performing short flights, which reveals their potential effect on the predatory interactions between species. The recorded unidirectional IGP is possibly due to *A. bipunctata* being larger and more voracious than *M. pygmaeus*.

It is known that some hemipteran and syrphid species are able to prey on *M. pygmaeus* in the absence and in some cases also in the presence of the extraguild prey (Jakobsen et al., 2004; Fréchette et al., 2007; Perdikis et al., 2014). As proposed by Lucas et al. (1998), it is possible that the extraguild prey density may affect the outcome of IGP. In the present experiment we recorded no IGP between *A. bipunctata* and *M. pygmaeus* when extraguild prey was abundant. The IG predator switched from feeding exclusively on aphids to feeding on IG prey when the encounter rate with the extraguild prey was low. These results fit with the assumptions of a model proposed by Křivan & Diehl (2005) that predict the coexistence of IG predators and prey. Further, the intensity of IGP is a function of the extraguild prey abundance. In the theoretical scenario proposed by Lucas et al. (1998), this kind of relationship between extraguild prey density and IGP is expected to occur for two predators that forage for prey randomly, in which their searching behaviour does not influence their chance of meeting.

Based on our previous experiments (data unpubl.) we know that under the same experimental conditions, when ten or twenty 2nd instar nymphs of *A. pisum* are offered to *M. pygmaeus* alone, the mirid bug consumed about five and seven prey items, respectively. In the present study, *M. pygmaeus* consumed about one aphid in the T10 and T20 experimental treatments. Moreover, we observed that *A. bipunctata* consumes the skins remaining after *M. pygmaeus* feeds on an aphid. Thus it is possible that the number of aphids consumed by *M. pygmaeus* was underestimated in our experiment. Nevertheless, in this system *A. bipunctata* could be considered the superior competitor.

Even if *M. pygmaeus* and *A. bipunctata* are not used together in aphid biological control, nowadays the propensity to include predatory mirids in biological control programs against aphids is more likely, especially in solanaceous plant crops (Pérez-Hedo & Urbaneja, 2014). Combining both predators may be the better strategy for crop protection because IGP was only substantial when the shared resource (aphids) was limited and because they differ in some important features. Coccinellids feed primarily on aphids and only opportunistically upon other prey (Hodek & Honěk, 1996). As a consequence, the release of the larvae usually does not result in the establishment of self-perpetuating populations (Powell & Pell, 2007) since coccinellids lay eggs in patches with aphid colonies at an early stage of development, and their developmental time is similar to the average duration of an aphid colony (Dixon & Hemptinne, 2003). Mirids are broad generalists and polyvoltine species and their density is not only influenced by the density of a specific prey (Harmon & Andow, 2004). For all these reasons coccinellids and mirids can exert a complementary action in biological control.

REFERENCES

ALOMAR O., RIUDAVETS J. & CASTAÑÉ C. 2006: *Macrolophus caliginosus* in the biological control of *Bemisia tabaci* on greenhouse melons. — *Biol. Contr.* **36**: 154–162.

BURGIO G., SANTI F. & MAINI S. 2005: Intra-guild predation and cannibalism between *Harmonia axyridis* and *Adalia bipunctata* adults and larvae: laboratory experiments. — *Bull. Insectol.* **58**: 135–140.

CASTAÑÉ C., ALOMAR O., GOULA M. & GABARRA R. 2004: Colonization of tomato greenhouses by the predatory mired bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*. — *Biol. Contr.* **30**: 591–597.

CHANG G.C. 1996: Comparison of single versus multiple species of generalist predators for biological control. — *Environ. Entomol.* **25**: 207–212.

COLFER R.G. & ROSENHEIM J.A. 2001: Predation on immature parasitoids and its influence of aphid population suppression. — *Oecologia* **126**: 292–304.

DE CLERCQ P., BONTE M., VAN SPEYBROECK K., BOLCKMANS K. & DEFORCE K. 2005: Development and reproduction of *Adalia bipunctata* (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen. — *Pest Manag. Sci.* **61**: 1129–1132.

DIXON A.F.G. & HEMPTINNE J.L. 2003: Ladybirds and the biological control of aphid populations. In Soares A.O., Ventura M.A., Garcia V. & Hemptinne J.L. (eds): Proceedings of the 8th International Symposium on Ecology of Aphidophaga: Biology, Ecology and Behaviour of Aphidophagous Insects. — *Arquipélago (Life & Marine Sci.) Suppl.* **5**: 1–10.

FRÉCHETTE B., ROJO S., ALOMAR O. & LUCAS E. 2007: Intraguild predation among mirids and syrphids: who is the prey? Who is the predator? — *Biocontrol* **52**: 175–191.

HARMON J.P. & ANDOW D.A. 2004: Indirect effects between shared prey: predictions for biological control. — *BioControl* **49**: 605–626.

HEMPTINNE J.-L., LOGNAY G., GAUTHIER C. & DIXON A.F.G. 2000: Role of surface chemical signals in egg cannibalism and intraguild predation in ladybirds (Coleoptera: Coccinellidae). — *Chemoecology* **10**: 123–128.

HINDAYANA D., MEYHOFER R., SCHOLZ D. & POEHLING H.M. 2001: Intraguild predation among the hoverfly *Episyrphus balteatus* de Geer (Diptera: Syrphidae) and other aphidophagous predators. — *Biol. Contr.* **20**: 236–246.

HODEK I. 1973: *Biology of Coccinellidae*. Academia, Prague & Dr. W. Junk, The Hague, 260 pp.

HODEK I. & HONEK A. 1996: *Ecology of the Coccinellidae*. Kluwer, Dordrecht, 464 pp.

HOLT R.D. & POLIS G.A. 1997: A theoretical framework for intraguild predation. — *Am. Nat.* **149**: 745–764.

INGELS B. & DE CLERCQ P. 2011: Effect of size, extraguild prey and habitat complexity on intraguild interactions: a case study with the invasive ladybird *Harmonia axyridis* and the hoverfly *Episyrphus balteatus*. — *BioControl* **56**: 871–882.

JAKOBSEN L., ENKEGAARD A. & BRODSGAARD H.F. 2004: Interactions between two polyphagous predators, *Orius majusculus* (Hemiptera: Anthocoridae) and *Macrolophus caliginosus* (Heteroptera: Miridae). — *Biocontr. Sci. Technol.* **14**: 17–24.

JANSSEN A., MONTSERRAT M., HILLE RIS LAMBERS R., DE ROOS A., PALLINI A. & SABELIS M.W. 2006: Intraguild predation usually does not disrupt biological control. In Brodeur J. & Boivin G. (eds): *Trophic and Guild Interactions in Biological Control*. Springer, Dordrecht, pp. 21–44.

KŘIVAN V. & DIEHL S. 2005: Adaptive omnivory and species coexistence in tri-trophic food webs. — *Theor. Popul. Biol.* **67**: 85–99.

LAW R. & BLACKFORD J.C. 1992: Self-assembling food webs: a global viewpoint of coexistence of species in Lotka-Volterra communities. — *Ecology* **73**: 567–578.

LUCAS E. 2005: Intraguild predation among aphidophagous predators. — *Eur. J. Entomol.* **102**: 351–364.

LUCAS E. & ALOMAR O. 2000: Susceptibilité de *Macrolophus caliginosus* (Heteroptera: Miridae) à la prédation intraguilde. — *IOBC/WPRS Bull.* **23**: 225–229.

Lucas E. & Alomar O. 2001: *Macrolophus caliginosus* (Wagner) as an intraguild prey for the zoophytophagous *Dicyphus tamaninii* Wagner (Heteroptera: Miridae). — *Biol. Contr.* **20**: 147–152.

Lucas E., Coderre D. & Brodeur J. 1998: Intraguild predation among three aphid predators: characterization and influence of extra-guild prey density. — *Ecology* **79**: 1084–1092.

Majerus M.E.N. 1994: *Ladybirds.* Harper Collins, London, 367 pp.

Moreno-Ripoll R., Gabarra R., Symondson W.O.C., King R.A. & Agustí N. 2014: Do the interactions among natural enemies compromise the biological control of the whitefly *Bemisia tabaci*? — *J. Pest Sci.* **87**: 133–141.

Omkar P.A. & Pervez A. 2005: Ecology of the two-spotted ladybird *Adalia bipunctata*: a review. — *J. Appl. Entomol.* **129**: 465–474.

Perdikis D., Lucas E., Garantonakis N., Giatropoulos A., Kitsis P., Maselou D., Panagakis S., Lampropoulos P., Paraskevopoulos A., Lykouressis D. & Fantinou A. 2014: Intraguild predation and sublethal interactions between zoophytophagous mirids (*Macrolophus pygmaeus* and *Nesidiocoris tenuis*). — *Biol. Contr.* **70**: 35–41.

Pérez-Hedo M. & Urbaneja U. 2014: Prospects for predatory mirid bugs as biocontrol agents of aphids in sweet peppers. — *J. Pest Sci.* **88**: 65–73.

Polis G.A. & Holt R.D. 1992: Intraguild predation: The dynamics of complex trophic interactions. — *Trends Ecol. Evol.* **7**: 151–154.

Polis G.A., Myers C.A. & Holt R.D. 1989: The ecology and evolution of intraguild predation: Potential competitors that eat each other. — *Annu. Rev. Ecol. Syst.* **20**: 297–330.

Powell W. & Pell K. 2007: Biological Control. In van Emden H.F. & Harrington R. (eds): *Aphids as Crop Pests.* CAB International, Wallinford, pp. 469–513.

R Core Team 2014: R: *A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, URL http://www.R-project.org/

Raak-van den Berg C.L., De Lange H.J. & Van Lenteren J.C. 2012: Intraguild predation behaviour of ladybirds in semi-field experiments explains invasion success of *Harmonia axyridis*. — *PLoS ONE* **7**(7): e40681.

Rondoni G., Onofri A. & Ricci C. 2012: Laboratory studies on intraguild predation and cannibalism among coccinellid larvae (Coleoptera: Coccinellidae). — *Eur. J. Entomol.* **109**: 353–362.

Rosenheim J.A. 1998: Higher-order predators and the regulation of insect herbivore populations. — *Annu. Rev. Entomol.* **43**: 421–447.

Rosenheim J.A., Wilhoit R. & Armer C.A. 1993: Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. — *Oecologia* **96**: 439–449.

Rosenheim J.A., Kaya H.K., Ehler L.E., Marois J.J. & Jaffee B.A. 1995: Intraguild predation among biological control agents: Theory and evidence. — *Biol. Contr.* **5**: 303–335.

Sakuratani Y., Matsumoto Y., Oka M., Kubo T., Fujii A. & Votani M. 2000: Life history of *Adalia bipunctata* (Coleoptera, Coccinellidae) in Japan. — *Eur. J. Entomol.* **97**: 555–558.

Santi F. & Maini S. 2006: Predation upon *Adalia bipunctata* and *Harmonia axyridis* eggs by *Chrysoperla carnea* larvae and *Orius laevigatus* adults. — *Bull. Insectol.* **59**: 53–58.

Ware R.L., Evans N., Malpas L., Michie L.J., O'Farrell K. & Majerus M.E.N. 2008: Intraguild predation of British and Japanese coccinellid eggs by the invasive ladybird *Harmonia axyridis*. — *Neobiota* **7**: 263–275.

Yano E. 2006: Ecological considerations for biological control of aphids in protected culture. — *Popul. Ecol.* **48**: 333–339.